SCIENTIFIC VALIDATION OF SIDDHA POLY HERBAL FORMULATION OF "*KANDANKATHARI CHOORANAM*" FOR ITS BRONCHODILATOR, ANTI-HISTAMINE AND ANTI-OXIDANT ACTIVITIES IN ANIMAL MODELS

The dissertation Submitted by

Dr. L. ILAVARASI

Reg no: 321612104

Under the Guidance of

Dr. R. KAROLIN DAISY RANI, M.D(S).,

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI - 600032

In partial fulfilment of the requirements

For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

CHENNAI – 106

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ABBREVIATIONS

ACh	Acetylcholine
ALT	Alanine Transaminase
ANOVA	Analysis Of Variance
AMP	Adenosine Monophosphate
AOM	Azoxymethene
AST	Aspartate aminotransferase
BHT	Butylated Hydroxy Toluene
BUN	Blood Urea Nitrogen
cAMP	Cyclic Adenosine Monophosphate
CD4+	Cluster of Differentiation 4
CMC	Carboxy Methyl Cellulose
COPD	Chronic Obstructive Pulmonary Disease
CPCSEA	Committee for the Purpose of Control & Supervision of Experimental Animals
DC	Differential count
DPPH	1-Diphenyl-2-Picryl-Hydraoxzyl
DTNB	Dithionitrobenzoic acid
E	Eosinophil
ECRHS	European Community Respiratory Health Society
ED50	Effective Dose
EDTA	Ethylene diamine tetra acetic acid
ESR	Erythrocyte Sedimentation Rate

FEV	Forced Expiratory Volume
FTIR	Fourier Transform Infrared Spectroscopy
FVC	Forced Vital Capacity
GINA	Global Initiative for Asthma
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
GSH	Glutathione
Hb	Haemoglobin
HL	Human Leukemic cell lines
HPLC	High Performance Liquid Chromatography
IAEC	Institutional Animal Ethical Committee
ICPOES	Inductively Coupled Plasma Optic Emission Spectroscopy
IL	Interleukin
ISAAC	International Study of Asthma and Allergies in Childhood
KKC	Kandankathari Chooranam
LTC4	Leukotriene C4
MTP	Mitochondrial Permeability Transition
MCV	Mean Corpuscular Volume
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OECD	Organisation for Economic Co-Operation Development
PCT	Pre-Convulsion Time
PCV	Packed Cell Volume

PEFR	Peak Expiratory Flow Rate
PGE2	Prostaglandin E2
RBC	Red Blood Corpuscles
SEM	Scanning Electron Microscope
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid Reactive Substances
TNF	Tumour Necrosis Factor
TLC	Thin Layer Chromatography
UV	Ultra Violet
WBC	White Blood Corpuscles
WHO	World Health Organization

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INTRODUCTION

1. INTRODUCTION

Siddha system is one of the earliest traditional medicine systems in the world. It is mainly practiced in the southern part of India. The term Siddha means an achievement. The practitioners of this medicine system are called Siddhars. They need to attain the physical, psychological, social and spiritual excellence to be able to treat people [(1)].

The roots of this system are intertwined with the culture of ancient tamil civilization. Herbal preparation are mostly preferred as the primary choice of medicines for various ailments, which is confirmed by the following literature quote [(2)].

Siddhars were the people who aimed for spiritual perfection to reach the ultimate goal of life. They were the greatest scientists who were supposed to have lived at a very early period. Siddhars were highly spiritual and intellectual personalities combined with supernatural powers that have the knowledge of healing art in curing many diseases of the mankind.

Siddha medicine aims at the perfection of health. Siddhars the concept of how a human body is exposed to various types of diseases.

The main principle is that the human body constituted with three basic humours named as Vadham, Pitham and Kabam which on derangement leads to diseased conditions or ill health and the deranged Vatham, Pitham, and Kabam are termed as "Three Doshas". [(3)]

The three humours are considered are three pillars of health and support the structure and functions of the body. These three humours are involved in regulating all the function of the body and maintain the balance in the physical, emotional and mental spheres.

The imbalance of humours especially kabam and vadham in Respiratory system modifies the air passage by secreting inflammatory mediators causing broncho constriction, suddenly leading to difficulty in breathing. Siddhars described this condition in literatures and named it as "Swasakasam or Iraippu". The symptoms of the disease Swasakasam are related to "Bronchial Asthma" [(4)].

According to World Health Organisation (WHO) Bronchial Asthma is inflammatory disease of the airways of the lungs. It is characterized by variable and

recurring symptoms reversible airflow obstruction and bronchospasm. Symptoms include episodes of wheezing, coughing, chest tightness and shortness of breath [5].

World Asthma day is an annual event organized by Global Initiative for Asthma (GINA) to world. World asthma day takes place on the first Tuesday of May [6].

As per ECRHS (European Community Respiratory Health Society) and ISAAC (International Study of Asthma & Allergies in childhood) the prevalence of Asthma increased steadily over the later part of the 20th century. About 300 Million people around the world wide suffer from Bronchial Asthma. India has been estimate to range 3-38% children and 2-12% in adults being the commonest chronic disorder among children [5].

A recent India Study on Epidemiology of Asthma Respiratory Symptoms and Chronic Bronchitis (INSEARCH) done with 85,105 Men and 84,470 Women from 12 Urban and 11 Rurel sites in india to be 2.05 % among those aged >15 years with an estimated national burden of 18 million asthmatics [7].

Although the development, course of disease and response to treatment are influenced by genetic determination, the rapid rise in the prevalence of asthma implies that environmental factors are critically important in terms of its expression.

Status Asthmaticus, secondary infection such as Bronchitis and Tuberculosis, Emphysema of lungs, later stage of Right Heart Failure called Chronic Cor Pulmonale, Bronchiectasis, Pneumothorax, Pneumo mediastinum are the complication of Bronchial Asthma.

Acute severe Asthma is termed as Status Asthmaticus is a medical emergency condition which is characterized by Tachycardia, Tachypnoea, Sweating, Pulses paradoxus and altered level of consciousness life threatening factors of Bronchial Asthma are central cyanosis, silent chest, severe hypoxaemia and altered consciousness [8].

According to who, mortality due to asthma is not comparable in size to the day to day effect of disease. Although largely avoidable. Asthma tends to occur in epidermics and affects young people. Worldwide the death from this condition has reached over 180,000 annually.

Asthma cannot be cured but could be controlled controlling asthma needs a good bronchodilator and also to control the hypersensitivity reaction. The famous bronchodilator drugs in market are Salbutamal, Theophylline, Isoprenaline etc… These drugs give only temporary relief to the patient with the reoccurrence of this disease and thereby causing adverse effect. For example, salbutamol causes some adverse effects such as muscle tremors, palpitation, restlessness and nervousness. Theophylline causes convulsion, shock and insomnia. Isoprenaline produce tachycardia [9].

Inhaled corticosteroids also increase the risk of cataracts (clouding of the eye lens) and osteoporosis (weakening of the bones) if taken for long periods of time [10].

Hence a safe and strong bronchodilator drug is needed. An internal medicine with remarkable efficacy is needed to administer to the affected individual.

Some of the popular Siddha drugs given for Bronchial Asthma are Mahavasantha kushmagaram, Poorna chendrodhayam, Swasakudori mathirai, Thalisathi chooranam, Pavala parpam, Arrakku thailam etc…… [4].

To control bronchial Asthma the world requires safety traditional fast acting Bronchodilators and potential drugs having anti-histamine activity to control the hypersensitivity reaction without any side effects.

In Siddha, purely herbal formulation provides biosafe and relieves the broncho constriction and other symptoms by their fast acting properties. They do not possess any adverse drug reactions. The author was interested in administering herbal preparation ***"KANDANKATHARI CHOORANAM"*** an effective bronchodilar or indicated for Bronchial Asthma in the siddha literature ***"AGATHIYAR ATTAVANAI VAGADAM"*** [11].

In this preparation most of the medicinal plants have bronchodilator activity, for example *Toddalia asiatica* (Milagaranai), *Solanum xanthocarpum* (Kandankathari), *Zingiber officinale* (Chukku) and *Piper nigrum* (Milagu). Anti histaminic plant in the preparation is *piper longum*.

Hence all these enriched plants used especially for many respiratory diseases are combined together in this herbal formulation ***"KANDANKATHARI CHOORANAM"*** will be used as bronchodilator in treating bronchial asthma (Swasakasam or Iraippu). Still now no scientific research works have been carried out on this herbal preparation. Therefore the author is interested to conduct Bronchodilator, Anti-Histamine and Anti-Oxidant activity of ***"KANDANKATHARI CHOORANAM"*** for Bronchial Asthma.

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

AIM

The aim of this dissertation is to establish the Scientific Validation of the Bronchodilator, Anti-Histamine and Anti-Oxidant property of ***Kandankathari Chooranam*** for Bronchial Asthma.

OBJECTIVES

The main objective of the present study is to highlight the efficacy of ***Kandankathari Chooranam*.** The following methodology was adopted to evaluate the drugs and its standardization studies

- Collection of various Siddha and modern literature relevant to the study.
- Identification of the drugs in this formulation.
- Preparation of ***Kandankathari Chooranam*** as per the classical Siddha literature.
- Physicochemical and phytochemical investigation of the test drug.
- Evaluate bio-chemical analysis of the test drug to derive acidic and basic radicals.
- To estimate the present of elements, functional groups and particle size through instrumental analysis of the trial drug.
- Evaluation of the Acute and 28 days repeated dose oral Toxicity of test drug according to OECD guidelines.
- Evaluation of pharmacological study of the drug through the following activities
- Evaluation of Bronchodilator activity
- Evaluation of Anti- histamine activity
- Evaluation of Anti -oxidant activity of ***Kandankathari Choornam.***

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

DRUG REVIEW

The trial drug "***Kandankathari Chooranam"*** was taken from the Siddha literature "***Agathiyar Attavanai Vagadam***" for treating Bronchial Asthma. The ingredients of the drugs are.

1. Kandankathari Root *(Solanum xanthocarpum)*
2. Araikeerai (*Amaranthus tristis*)
3. Chukku *(Zingiber officinale)*
4. Thippili *(Piper longum)*
5. Milagu (*Piper nigrum)*
6. Milagaranai pattai (*Toddalia asiatica*)
7. Sugar *(Saccharum officinarum)*
8. Ghee

3.1. GUNAPADAM REVIEW

KANDANKATHRI

Scientific Name **:** *Solanum xanthocarpum*

Synonyms **:** *Solanum surattense* .Burm. f.
Solanum virginianum [(12A)].

Vernacular Names

Tamil : *Kandangkattari*

English : Wild egg plant, Bitter sweet woody nightshade, Yellow berried night shade

Telugu : *Nela Mulaka-Vakudu*

Malayalam : *Vellottuvalutina*

Hindi : *Kateli*

Sanskrit : *Kanta-karika*

Kannadam : *Nela-gulla*

Parts Used : Leaf, flower, root, fruit, seed

Properties:

Suvai (Taste) : *Karppu*

Thanmai (Nature) : *Veppam*

Pirivu (Bio- Transformation) : *Karppu*

Actions:

- Expectorant
- Diuretic
- Carminative

General Characters:

"காச சுவாசங் கதித்தகூய மந்தமனல்
வீசுசுரஞ் சன்னி விளைதோடம் - ஆசுறுங்கால்
இத்தரையு ணிற்கா எரிகாரஞ் சேர்க்கண்டங்
கத்திரியுண் டாமாகிற் காண்."

- அகத்தியர் குணபாடம்

Indications : It cures Cough, loss of appetite and Asthma.

Therapeutic Uses :

- A decoction of the leaves and roots with long pepper in the dose of half to one ounce with honey was an excellent mixture used in chronic bronchitis and asthma.
- Juice of berries are beneficial in sore throat.
- Leaf juice in a combination with black pepper prescribed in Rheumatism.
- Root decoction in combination with *Tinospora cordifolia* was useful in cough and fever.

ARAIKEERAI

Scientific Name : *Amaranthus tristis*

Synonyms : *Spleen amaranth*

Amaranthus dubius [12B].

Vernacular Names

Tamil : Araikeerai

English : Pig weed amaranth

Spanish : Bledo de jamaica

French : Brede de Malabar

Parts used : Whole plant

Properties :

Suvai (Taste) : Inippu (Sweet)

Thanmai (Nature) : Veppam

Pirivu (Bio – Transformation) : Inippu (Sweet)

Actions:

- Stimulant
- Aphrodisiac
- Laxative
- Diuretic

General Characters:

"காய்ச்சல் குளிர்சன்னி கபநோய் பலபிணிக்கும்
வாய்ச்ச கறியாய் வழங்குங்காண் - வீச்சாய்க்
கறுவுமோ வாயுவினங் காமமிக வுண்டாம்
அறுகீரை யைத்தின் றறி".

- பதார்த்த குண சிந்தாமணி

Indications:

- It cures Anaemia, Haemorrhage and Constipation

Therapeutic Uses:

- Leaves in general are recommended as a good food with medicinal properties for young children, lactating mothers and for patients with Fever, Haemorrhage, anaemia, Constipation and Kidney complaints.
- The whole plant is used as a medicine against stomach ache.

CHUKKU

Botanical name : *Zingiber officinale*

Synonyms : *Nagaram, Atagam, Aartharagam, Chowpannaum, Verkombu, Nava suru, Ullarntha inji, Vidam moodiya amirtham***.** (12C)

Vernacular names

Tamil	: Chukku
English	: Dried ginger
Telugu	: Sonti
Malayalam	: Shukka
Kannadam	: Ona shunti or Sunti
Sanskrit	: Nagaram
Hindi	: Sonth

Part used **:** Dried Rhizome

Properties

Suvai (Taste)	: Karppu
Thanmai (Nature)	: Veppam
Pirivu (Bio-Transformation)	: Karppu

Actions

- Stimulant
- Stomachic
- Carminative

General Characters

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர் - வாலகப
தோடமதி சாரந் தொடர்வாத குன்மநீர்த்
தோடம்ஆ மம்போக்குஞ் சுக்கு."

- அகத்தியர் குணபாடம்

Indications

- Dried ginger was used for Indigestion, Gastric irritation, Anal diseases, Asthma, Cough, Diarrhoea, Sinusitis, Anaemia and Fever.

Therapeutic uses

- A pinch of dried ginger powder with cow's milk is useful in loss of appetite.
- Dried ginger powder with sugarcane juice reduces burning sensation of the stomach.
- Dried ginger with sugar candy powder taken with tender coconut in morning and evening for dyspnoea and chest pain after heavy working.
- Dried ginger decoction is useful for poisonous type of fever.
- Chewing a piece of dried ginger helps in relieving the tooth ache.[13A]

THIPPILI

Botanical name **:** *Piper longum*

Synonyms **:** *Pippli, Aadhi,Kaaman, Sowndi, Kanam, Saram, Koli, Ambu, Aathimarunthu, Kanai.*[12D].

Vernacular names:

Tamil	: Thippili
English	: Long pepper
Telugu	: Pippilu
Malayalam	: Thippili
Kannadam	: Hippili
Sanskrit	: Pippali
Hindi	: Pipar

Part used : Dried fruit and Roots

Properties

Suvai (Taste) : Karppu

Thanmai (Nature) : Veppam

Pirivu (Bio- Transformation) : Karppu

Actions

- Carminative
- Stimulant
- Expectorant
- Antiseptic
- Febrifuge

General characters

"ஈளை யிருமை லிரைப்புப் பசப்பிணிகள்
மாளன வொழியாமல் வாட்டுமே - யாளுமுறை
பாங்கா யறிந்துசெய்வீர் பண்டிதத்தைப் பண்டிதரே
வேங்கைவாய்ப் பாங்கணை மெய்.

- தேரன் வெண்பா

Indications

- It relieves Kapha related diseases and strengthens the body.

Therapeutic uses

- Powered long pepper with honey will relieve cough, cold, asthma, hoarseness and hiccough.
- Long pepper powder with honey and betel leaf juice cures fever.
- A mixture of long pepper, long pepper root, black pepper and ginger in equal proportions is used to relieve colic and flatulence.
- Powered form of long pepper seeds with ghee is used for its aphrodisiac action [13B].

MILAGU

Botanical name : *Piper nigrum*

Synonyms : *Malayali, Maasam, Sarumabandam, Kaayam, Kalinai, Miriyal, Thirangal* [12E].

Vernacular names

Tamil	:	Milagu
English	:	Black pepper
Telugu	:	Miriyalu
Malayalam	:	Kurumilagu
Sanskrit	:	Maricha
Hindi	:	Kali-mirch

Part used : Dried fruits

Properties

Suvai (Taste)	: Kaippu, Karppu
Thanmai (Nature)	: Veppam
Pirivu (Bio- Transformation)	: Karppu

Actions

- ❖ Carminative
- ❖ Stimulant
- ❖ Anti-vadha
- ❖ Antidote

General characters

"சீதசுரம் பாண்டு சிலேத்மங் கிராணிகுன்மம்
வாதம் அருசிபித்தம் மாமூலஓது - சன்னி
யாசமபஸ் மாரம் அடன்மேகம் காசமிவை
நாசங் கறிமிளகி னால்".

- அகத்தியர் குணபாடம்

Indications

- It cures Anaemia, Gastric ulcer, Giddiness, Diarrhoea, Dysentery, Vomiting, Anal fissure and Cataract.

Therapeutic uses

- Dried unripe fruits are prescribed in cholera, dyspepsia, flatulence and various gastric ailments.
- Powdered black pepper with onio and salt is made into a paste, and this mixture is applied on the scalp to cure alopecia and also to increase the hair growth.
- Black pepper paste is applied externally for boils.
- Powder of black pepper is used as tooth powder [13C].

MILAGARANAI

Botanical names : *Toddalia asiatica*

Synonyms : *Toddalia aculeate pers*

Paullinia asiatica [12F].

Vernacular names

English	:	Forest pepper
		Wild orange tree
		Lopez-root tree
Malayalam	:	Milakaranai
Sanskrit	:	Kanchana
Hind	:	Jangli – kalimirch
Telugu	:	Konda-kashinda
Kanadam	:	Kada-hakukare
Bengali	:	Dahana

Parts used : Leaf, Bark, Root.

Properties

Suvai (Taste)	: Thuvarppu
Thanmai (Nature)	: Thatpam
Pirivu (Bio – Transformation)	: Kaarppu

Actions

- Stimulant
- Tonic
- Carminative
- Diaphoretic
- Antiperiodic

General characters:

"ஐயம கற்றும் அசீரணவா தம்போக்குஞ்
செய்யபித்த சூலைகளைத் தீர்க்குங்காண் - பையவரும்
ஈளை இருமல் இரைப்புப்பு சந்தொலைக்கும்
நாளு மிளகரணை நன்று".

- அகத்தியர் குணபாடம்

Indications :

- It cures Indigestion, Cough, Bronchial asthma and Stomach ailments.

Therapeutic uses:

- The fruits are used as a cough remedy.
- The leaves are used for lung disease and rheumatism.
- The roots in the treatment of indigestion and influenza.
- The root bark is used medicinally as a tonic and for stomach ailments [14].

ASSOCIATED DRUGS:

GHEE (Nei)

Butter is cleaned and heated in a vessel. When it melts either the leaves of *Moringa olifera* or the bettle leaves are added and filtered immediately, since it is difficult to filter after cooling.

If Ghee is preserved in a good vessel, it won't spoil upto months. Cow's ghee is slightly yellowish in colour. Ghee should be forbidden. It is good to consume the melted ghee and diluted butter milk. The following lines stress these points:

"நீர்சுருக்கி மோர்பெருக்கி நெய்யுருக்கி யுண்பவர் தம்

பேருரைக்கிற் போமே பிணி".

- சித்த மருத்துவாங்கச் சுருக்கம்[14]

General properties:

"நெய்யுண வுண்டவை நேர்வுறச் செய்துமேன்

மெய்யையுந் திண்ணிய மேருவெனச் செய்யும்.

- குணபாடம் தாதுசீவவகுப்பு [15].

When ghee taken in required quantities along with usual diet, it helps in proper digestion and utilization of the diet and gives strength and vigor to the body.

Cow's ghee:

It controls thirst, Vomiting, Excessive Pitha, Burning sensation of the stomach, Pitha hi-cough, Abdominal pain, Dryness, Pricky heat, Cough, Hypermotility of the gut, Weakness of bones, Piles etc.

Medicinal uses:

- When ghee is mixed in hot rice and eaten, it enhances the healing of peptic ulcer. It also stimulate bone marrow growth. Ghee should be eaten only after melting (liquid form)
- Dried ginger, Pepper, Cuminum seeds are fried, powdered and taken along with ghee for indigestion and dysentery.
- For curing stomach pain, the ghee is taken with boiled rice water and if it is taken with palm sugar candy, it cures body heat and whooping cough.
- Ghee is also used as an adjuvant for many parpams, Chendhoorams, Leghium, and Thailams .
- By taking ghee bath, the burning sensation, pitha, Unconsciousness, Haematemesis etc. are cured.[16].

SUGAR (*SARKARAI*)

Botanical name : *Saccharum officinarum*

Synonyms : *Punarpoosam, Ikku, Vei* [12G].

Vernacular names

Tamil	:	Karumbu
English	:	Sugarcane, Noble cane
Telugu	:	Cheruku
Kannada	:	Karinpa
Malayalam	:	Khabbu

Parts used:

Sugarcane juice, Sugar, Root

Properties and uses

Taste(Suvai) : Inippu (Sweet)

Nature(Potency) : Thatpam (Cold potency)

Division(Pirivu) : Inippu (Sweet)

Action

- Demulcent
- Antiseptic
- Stimulant
- Diuretic
- Nutrient

General characters:

"சீனிச் சர்க்கரைக்குத் தீராத வன்சுரமுங்
கூனிக்கும் வாதத்தின் கூட்டுறவும் - ஏனிற்கும்
வாந்தி யொடுகிருமி மாறாத விக்கலுமே
போந்திசையை விட்டுப் புரண்டு".

- அகத்தியர் குணபாடம்

Indications:

- It can be used as an adjuvant for several medicine
- It cures vomiting.

Therapeutic uses:

- ❖ It cures Fever, Vomiting and Hiccough.
- ❖ It cures Vadha fever, Common cold and Sinusitis.
- ❖ Paste of sugar with bee wax is used to treat acne.
- ❖ It cures eye diseases.

3.2. BOTANICAL REVIEW

KANDANKATHARI (*Solanum xanthocarpum)*

Scientific classification (17)

Kingdom	: Plantae
Class	: Dicots
Order	: Solanales
Family	: Solanaceae
Genus	: *Solanum*
Species	: *xanthocarpum*

Distribution:

Grown abundantly in India Common in road sides waste places and along railway lines throughout India.

Description :

A prickly diffuse perennial herb. Numerous branches. Leaves ovate or elliptic sinuate or subpinnatifide glabrescent, with many straight spines. Flowers borne in few flowered lateral cymes. Corolla blue, lobes shallow, Fruits globose, glabrous berries, Whitish and green blotched, yellow when ripe. Seeds glabrous.

Flowers and fruits during March – July (18A)

Parts used:

Leaf, Flowers, Fruits, Seeds, Root.

Chemical Constituents:

Carpesterol, Gluco-alkaloid solanocarpine, Solanine-S, Solasodine, Solasonine, Solamargine, Cycloartanol, Cycloartenol, Stigmasterol, Campesterol, Cholesterol, Sitosteryl-glucoside, Stigmasteryl-glucoside, Solasurine [19].

Properties:

The plant is Acrid, Bitter, Digestive, Alternative, Astringent, Expectorant, Diuretic, Carminative, Hydragogue, Antifilarial and Hepatoprotective.

Uses:

- Root decoction used as febrifuge, effective diuretic and expectorant.
- The whole plant is used traditionally for curing various ailments.
- Decoction of the plant is used in gonorrhea.
- Paste of leaves is applied to relieve pain [20].

***ARAIKEERAI* (*Amaranthus tristis*)**

Scientific classification [21]

Kingdom	: Plantae
Class	: Dicots
Order	: Caryophyllales
Family	: Amaranthaceae
Genus	: *Amaranthus*
Species	: *tristis*

Distribution:

- A.tristis is Native to South America, Mexico and the West Indias and naturalized or invasive in the USA, central and Southwest Africa, Asia.
- It is cultivated in many countries especially in Africa, but it can be difficult to know whether its presence in a country is only as a cultivated plant or as a naturalized plants.

Description:

- Annual herb, 10-100 cm tall glabrous or sparsely pubescent in distal parts. Stems erect, green, branched 0.3-1mm
- Leaves petiole of proximal leaves equaling or longer than blade, becoming Shorten distally. Stipules absent.
- Inflorescences terminal panicles and axillary spikes. Panicles erect or often drooping, green, dense branched, leafless at least distally.
- Bracts lanceolate, shorter than 2mm pistillate flowers tepals 5, oblong-spathulate to oblong, not clawed, very shortly mucronate, yellowish.
- Seeds dark reddish brown to black, lenticulate, 0.8-1mm diameter, shiny, smooth.

Chemical constituents:

Acifluorfen, atrazine, bensulfuron, butachlor, chlorthal-dimethyl, dimethametryn, diphenamid, diuron, glyphosate, metribuzin, oxadiazon, oxyfluorfen, paraquat, pendimethalin, propanil and trifluralin.

Parts used:

Whole plants

Properties:

The plant is Acrid, Laxative, Diuretic.

Uses:

- Leaves in general are recommended as a good food with medicinal properties for young children.
- Seeds and oil have fiber which contributes to lower cholesterol and risk of constipation.
- It is rich in phytosterols and also known for lowering cholesterol.
- The leaves are high in vitamin C, Vitamin A and Folate.

CHUKKU (*Zingiber officinale)*

Scientific classification [22]

Kingdom : Plantae
Class : Monocholamydeae
Order : Zingiberales
Family : Zingiberaceae
Genus : *Zingiber*
Species : *officinale*

Distribution:

Cultivated throughout India, run wield in some places in the Western Ghats.

Description

A Slender, perennial rhizomatous herb; leaves linear, sessile, glabrous; flowersyellowish green in oblong, cylindrical spikes, en-sheathed in a few scarious, glabrous bracts; fruits along capsules. The rhizomes are white to yellowish brown in colour, irregular branched, somewhat annulated and laterally flattened. The growing tips are covered over by a few scales. The surface of the rhizome is smooth and if broken a few fibrous elements of the vascular bundles project out from the cut ends [23].

Parts used:

Rhizome

Chemical Constituents

The characteristic fragrance and flavor of ginger result from volatile oil that compose 1-3%of the weight of the fresh of ginger, primarily consisting of zingerone, shogaols and gingerols with gingerol as the major pungent compound. Zingerone is produced from gingerols during drying, having lower pungency and a spicy-sweet aroma [24].

Properties:

The dry ginger is Acrid, Thermo-genic, Emollient, Appetiser, Laxative, Stomachic, Stimulant, Rubefacient, Aphrodisiac, Expectorant, Anthelmintic and Carminative.

Uses:

It is useful in Dropsy, Otalgia, Cephalic, Asthma, Cough, Colic, Diarrhoea, Anorexia, Dyspepsia, Cardiopathy, Cholera, Nausea, Vomiting, Elephantiasis and Inflammation. It is also much used in several domestic preparations [25].

THIPPILI* (*Pipper longum)

Scientific classification [26]

Kingdom	: Plantae
Class	: Magnolipsida
Order	: Piperales
Family	: Piperaceae
Genus	: *Piper*
Species	: *longum*

Distribution

This plant mostly occurs in hotter parts of the India from central Himalayas to Assam up to lower hills of the west Bengal and evergreen forests of western Ghats as wild and also cultivated in north east and south. It grows in Kurinji nilam.

Description of the plant

A slender aromatic climber and leaves alternative, lower ones broadly ovate cordate, upper ones oblong, oval, all entire 5 to 7 nerved leaves; male spikes longer, slender, 2.5 to 7.5 cm long. Female spikes short, cylindrical, 1.5 to 2.5 cm long, 5 to 7 mm thick. Fruit greenish- black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis. Surface rough and composite;

broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis. Odour is aromatic; taste is pungent producing numbness of the tongue [27A].

Parts used:

Roots, Dried spikes.

Chemical constituents:

Volitale Oil, Resin, Piperin, Piperlongumine, Piperlatin, Brachyamide A, Brachyamide B, Brachystine, Sterols, Glycosides [28].

Properties:

The roots are bitter, Tonic, Diuretic, Purgative, Expectorant, Stomachic, Digestive and Emmenagogue.

Uses:

- They are useful in Gout, Dyspepsia, Stomachalgia and Spleenopathy.
- It cures Anorexia, Dyspepsia, Asthma, Bronchitis, Hiccough, Epilepsy, Fever, Gonorrhoea, Haemorrhoids and Lumbago [29].

MILAGU (Piper nigrum)

Scientific classification [30]

Kingdom	: Plantae
Class	: Dicot
Order	: Microembreye
Family	: Piperaceae
Genus	: *Piper*
Species	: *nigrum*

Distribution

The plant cultivated in the hotter and moist parts of India, in evergreen forest up to 1,500 meters.

Description

Climbing perennial shrubs, rooting at the nodes, leaves are cordate or round based; flowers minute in spikes usually dioeciously. Fruiting spikes very variable in length, fruits ovoid or globose one seeded berries, bright red when ripe, seeds are globose, albumin hard and testa thin greyish-black to black, perisperm hard, wrinkled and white, 0.4 to0.5 cm in diameter; odour aromatics, taste pungent.

Flowering occurs in the rainy season and fruits ripening in the autumn season (December to April) [27B].

Parts used:

Dried fruits.

Chemical Constituents

Piperine, chavicine, Piperettine, Piperoline A&B, Trichostachine, N-trans-feruloyl piperidine, Feruperine, Citrohellol, Arginine, Piperolic acid, Serine, Ascorbic acid, Carotene [31].

Properties:

The fruits are Acrid, Bitter, Anthelmintic, Carminative, Aphrodisiac, Antiperiodic, Diuretic, Digestive, Emmenagogue, Stimulant and Stomachic.

Uses:

- They are useful in Arthritis, Asthma, Fever, Cough, Dysentry, Dyspepsia, Hiccough, Haemorrhoids and Dermatopathy [32].

MILAKARANAI (Toddalia asiatica)

Scientific classification [33]

Kingdom	: Plantae
Class	: Dicot
Order	: Sapindales
Family	: Rutaceae
Genus	: *Toddalia*
Species	: *asiatica*

Distribution:

Found in sub-tropical Himalayas, from Kumaon eastwards to Assam, Khasi hill and throughout the Western Peninsula.

Description:

A throny, scandent shrub, Leaves 3-foliolate; leaflets sessile, elliptic, obovate, oblongor lanceolate, crenulate, coriaceous, Flowers white, borne in axillary, compressed cymes; calyx glandular. Fruits globose, 3-5-grooved, orange- coloured, 3-5-celled. Seeds solitary in each cell.

Flowers during February- March and fruits during May- June.

Parts used:

Leaf, Bark, Root.

Chemical constituents:

Berbapten, norbraylin, 5,7,8-trimethoxycoumarin, dictamnine, luvangetin and robustin(stem); hexacosanoic acid, arnottainamide, dihydroavicine, 8-acetoxy, aculeatin,diosmin, phnanthridine, isopimpinellin, pimpinellin, toddaculine, toddanol, toddanone, and toddasin .

Properties:

Plant leaves and stem have bitter taste, minty and warming- nature and considered antiphlogistic and analgesic in nature.

Root bark of the plant is Antimalarial, Antiperiodic, Antipyretic, Tonic and Carminative.

Uses:

Toddalia asiatica is widely used by many African tribes

- It is used for treatment of Malaria Cough and Influenza.
- It is used as a pain killer.
- Leaf essential oil is used in relieving Rheumatic arthritis, Sprains and Contusions.
- Intercostals neuralgia, Cough, Malaria, Dysentery and Gastralgia
- It is also used as an antidote against poisonous Snakebite, Nausea, Bronchitis, Wounds. Contaminated Ulcers, Epilepsy, Gonorrhea and General Debility [(34)].

ASSOCIATED DRUGS:

GHEE

English name:

Ghee, Clarified butter.

Nutritional profile:

- Ghee is relatively high in calories, containing 112 calories per tablespoon serving, A serving contains 12.7 grams of fat, minimal amounts of protein and no carbohydrates dietary fiber or sugars.
- Ghee is high in saturated fat with 7.9 grams per serving.

Vitamins and Mineral content:

- It contains only a minimal amount of calcium 1mg/tsp Ghee also contains 108 micrograms of vitamin A, which is a significant amount for such a small serving size.
- 1 tsp provides 12 and 15% of the recommended daily intakes of vitamin A for men and women, respectively [35].

SUGAR (Saccharum officinarum)

Scientific classification

Kingdom	: Plantae
Class	: Monocotyledons
Order	: Poales
Family	: Poaceae
Genus	: *Saccharum*
Species	: *officinarum*

Distribution:

Sugar cane is indigenous to tropical south and southeast Asia.

Description:

It is a topical, perennial grass that forms lateral shoots at the base to produce multiple stems, typically three or four meters high and about 5 cm diameter and once harvested the stalk will regrow allowing the plant to live for between 8 to12 years. The stem grows into cane stalk, which when mature constituents approximately 75% of entire plant. A mature stalk is typically composed of 11 – 16% fiber, 12- 16% soluble sugars, 2-3% non-sugar and 63-73% water. The leaves are grow from the nodes of the stem, arranged in two rows on either side of the stem. The leaves are tubular and blades like, thicker in the centers than at the margins panicle which possesses two spikelet and seeds protected by husks covered in silky hair.

Parts used:

Roots, Stems [36A]

Chemical constituents:

Sucrose is the product of the sugar cane juice. The juice yielded flavones diosmetin-8-C-gulcoside, vitrexin, schaftoside, isoschaftoside and 4',5'-dimethyl-luteolin-8-C glucoside [37].

Properties:

The roots are cooling and diuretic. The stems (sugar cane) are Sweet, Cooling, Emollient, Laxative, Cardio- tonic, Diuretic, Galactogogue, Aphrodisiac, Expectorant, Haemostatic and tonic.

Uses:

- The roots are useful in Uropathy.
- They are useful in Dipsia, Fatigue, Leprosy, Gastropathy, Cardiac, Debility, Hamatemesis, Cough, Bronchitis, Anaemia, Ulcers of the skin and mucous membrane. Seminal weakness, Emaciation and general debility [36B].

3.3. SIDDHA ASPECT OF THE DISEASE

SWASAKASAM (BRONCHIAL ASTHMA)

Other Names

- *Iraippu*
- *Izhuppu noi*
- *Swasam*
- *Thoivu*
- *Eelai*
- *Suram*
- *Iraippirumal*

Nature of the disease

Swasakasam arises with severe chest tightness leading to difficulty in inspiration and expiration of the air (i.e. dyspnoea). In addition to difficulty in breathing while exhaling the air, expiratory noise will be produced resembling the sounds of musical instruments like flute, veena, etc., are heard obviously. Further if hard attempts are made to expel the phlegm, it results in vain.

Genesis of the disease

"கால்பெருக் குணவுபொருள் தண்ணீர் மாறல்
கருதிருமல் மிகல்வாந்தி குளிர்ந்த காற்று
மால்செய்து நாள்தோறும் வருந்துங் காய்ச்சல்
மந்தன முயிர்நிலை யிலடிகள் தாங்கல்

ஏல்சீத பேதிவிட பாண்டு புகைகள்
இலகிய நெல்லாதிமணிச் சுனையுட் செல்லல்
மேல்வழியிற் சிலவரினு மிரைப்பாம் நோயு
முனிவர்கள் விளம்பினாரே"

- கையெழுத்துப்பிரதி

Swasakasam is caused due to the following factors such as

- Ingestion of allergic food stuffs
- Allergy inducing activities such as exposed to cool climates.
- Immunity deprivation
- Intake of diet which increases kapha.
- Grass, rice and ragi also triggers the signs and symptoms
- Symptoms may also develop due to inhalation of foul smelling substances.

Prodromal symptoms

"மார்பில் விளாவிரண்டில் மண்ணுனமிகு நெரியில்
சேர்ந்து வலித்தல் திணறல் - தார்மூச்சு
உப்பல் வயிற்று லுருவது முற்குறியாச்
செப்பிரைப்பு நோய்க்குதனைத் தேர்"

- யூகி வைத்திய சிந்தாமணி[38]

Generally the prodromal symptoms and intensity of the disease will be recognised earlier by chronic asthmatics. While taking unsuitable food and while inhaling the chill air the patient develops rhinitis, sneezing, chest discomfort, chest tightness, pain, difficulty in normal breathing, pain in para vertebral region with dyspnoea, distension of abdomen and excess sweating.

Types of *Swasakasam*

Swasakasam (Iraippu) has been classified into 5 types.

Of these, first four types are based on *kuttram* and the final one is based on intensity of breath. They are as follows

1. *Vali Iraippu*
2. *Iya Iraippu*
3. *Iyavali Iraippu*
4. *Mukkutra Iraippu*
5. *Melnokku Iraippu*

Apart from this further it is also classified into another 5 types.

"சிறுபே றிரைப்பு திணறல் மந்தாரம்
வருமே லிரைப்புந் தின்மாண்பு"

- யூகி வைத்திய சிந்தாமணி

They are as follows

- *Sittru Iraippu*
- *Per Iraippu*
- *Thinaral Iraippu*
- *Mandhara Iraippu*
- *Mael Iraippu*

Signs and symptoms

"வன்மையாய் கோழைகட்டி இருமி வீழும்
மாநாகம் போலவே வாங்குஞ் சுவாசம்
திண்மையாய்ச் சேருமுண்டா மடிக்க டிக்குஞ்
சீரண மிலாமலே வயிறு மூதும்
நன்மையாய் நாசியது தணல்போ லாகும்
நலிந்துடம்பு வற்றி வருங் குரலுங் கம்மும்
உண்மையா யுண்ணாக் கிலூறுங் கேணி
யுழந்துமே சுவாசகா சத்தி னொப்பே"

- யூகி வைத்திய சிந்தாமணி[38A]

Vali iraippu noi

Vatha dosha gets aggravated due to ingestion of food that is not easily digested, wandering under hot sun rays, eating tubers. Due to increased *vatha dosha* the patient may feel condition as if nothing is inside the chest. Inspite of all these conditions patient doesn't experience severe illness and this condition is curable. *Vali iraippu* is also mentioned as "*Soothira swasam".*

Kabha swasam

Kapha swasam is caused due to increased *kapha dosha* because of taking foods which increase *kapha* and also roaming in chill air. It produces nasal congestion, rhinitis, chest tightness, inability to breathe.

Sometimes constricted type of chest pain may aggravate to the extent as if the patient dies of inability to breath. When the patient mildly attempts to cough and expectorates some mucus relief occurs for some extent. When the patient does not cough and expel the mucus dyspnoea occurs and patient is unable to lie on bed, makes him to stand.

Sweating on forehead, blackening of face, chillness of limbs, dryness of tongue, shivering of body, dyspnoea, inability to sleep are the associated symptoms of this disease. It is also known as "*Thamaraga swasam".*

Iyavali iraippu

In this condition both *kapha dhosam* and *vatha dhosam* are dearranged together and causes the following symptoms. Symptoms of this type will be very severe and the derangement of *vatha dosha* combines along with *udhana vaayu*.

Clinical features of this type are dyspnoea, inability to inspirate and expirate air, constipation, abdominal distension, dryness of tongue, redness and painful eyes, shivering of body, giddiness, excessive sleep, incoherent talk, etc., this condition is also called as "*Vichinna Swasam".*

Mukkuttra iraippu

In this condition, all the three doshas gets deranged at once and *udhana, abanan, viyanan, samanan* get deranged one by one which in turn affects the seven major elements of the body. It is life threatening type of asthma.

The prodromal symptoms are Shivering of body, dyspnoea, depression, breathing like cow's breathing, chest tightness and pain, constipation, oliguria, pain all over the body, stammering and excessive sweating over the forehead. This is also called as "*Thinaral Iraippu*".

Maelnokku Iraippu

If any of the above mentioned disease continues for many days without response to treatment, then the upward directional *udhana vaayu* loses its strength and in such a situation, expiration may not be possible. The patient tends to develop dyspnoea with prominent eyeball. There may be dryness of mouth. Patient may be unable to speak; he may appear astonished and will not lie down on the bed he may look upward he may also attempt to exhale by his opened mouth. If proper treatment is given at this stage he may survive. Otherwise he may fall unconscious with darkening of face and may die with mouth open[38].

Other factors affecting the disease

- Eating foods which will induce excessive *kapha.*
- Exposure to chill air.
- Living in the mountains
- Walking in the cold climate.

Pulse

"கபமல்லாது காச சுவாசம் வாராது" [39].

- *Kaba Nadi*
- *Vathakaba Nadi*
- *Kapha pitha Nadi* are the classical pulse for *Swasakasam*.

Sputum

- If the sputum is found excessive in quantity, light weight and foamy, it is considered that the disease gets developed due to *Kapha dosham*.
- If the sputum is black in colour, hard and with smell of flesh, it will denote *Kapha dosham*.
- If it is found white like pus and mixed with yellow colour, it will denote P*itha dosham.*[40].

3.4. MODERN ASPECT OF THE DISEASE

BRONCHIAL ASTHMA

Introduction

Asthma, the word was derived from Greek word. The term **"ASTHMA"** in Greek means breathless or breathe with open mouth.

Asthma is defined as a chronic inflammatory disorder of the airways, characterised by reversible airflow obstruction causing cough, wheeze, chest tightness and shortness of breath. Inflammation of the bronchial wall involving eosinophil, mast cells and lymphocytes, together with the cytokine and inflammatory products of these cells, induces hyper-responsiveness of the bronchi so that they narrow more readily in response to a wide range of stimuli. Narrowing of the airway in usually reversible, but in some patients with chronic asthma the bronchial wall inflammation may lead to irreversible obstruction of airflow.

Asthma is thought to be caused by a combination of genetic and environmental factors. Environmental factors include exposure to air pollution and allergens. Other potential triggers include medications such as aspirin and beta blockers.

Diagnosis is usually based on the pattern of symptoms, response to therapy over time, and spirometer. Asthma is classified according to the frequency of symptoms, forced expiratory flow rate. It may also be classified as atopic or non-atopic, where atopy refers to a predisposition toward developing a type 1 hypersensitivity reaction.

Epidemiology

The prevalence of asthma increased steadily over the later part of the century first in the developed and then in the developing countries. Current estimates suggest that asthma affects 300 million people world-wide and additional 100 million persons will be diagnosed by 2025 In India,15-20 millions are asthmatics. About 2,50,000 annual deaths.

In 2015, 358 million people globally had asthma, up from 183 million in 1990.It caused about 397,100 deaths in 2015, most of which occurred in the developing world. It often begins in childhood.

Epidemiological studies suggest that the multiple genetic and environmental factors contribute to the causation of asthma, a clinical condition that is viewed as a cluster of related disorders to smooth muscle hypertrophy. (41)

Signs and symptoms

Asthma is characterized by recurrent episodes of wheezing, shortness of breath, chest tightness and coughing. Sputum may be produced from the lung by coughing but is often hard to bring up. During recovery from an attack, it may appear pus-like due to high levels of white blood cells called eosinophills.

Symptoms are usually worse at night and in the early morning or in response to exercise or cold air. Some people with asthma rarely experience symptoms, usually in response to triggers, whereas others may have marked reactivity and persistent symptoms.

Associated condition

A number of other health conditions occur more frequenty in those with asthma, including gastro-esophageal reflux disease (GERD), rhinosinusitis and obstructive sleep apnea. Psychological disorders are also more common, with anxiety disorders occurring in between 16-52% and mood disorders in 14-41%. However, it is not know whether asthma causes psychological problems or psychological problems lead to asthma. Those with asthma, especially if it is poorly controlled, are at increased risk for radio contrast reactions.

Causes of asthma

No single cause has been identified for asthma. Instead, researchers believe that the breathing condition is caused by a variety of factors.

These factors include:

- **Genetics:** If a parent has asthma, Children more likely to develop it.
- **History of viral infections:** People with a history of viral infections during childhood are more likely to develop the condition.
- **Hygiene hypothesis:** This hypothesis proposes that babies aren't exposed to enough bacteria in their early months and years. Therefore, their immune systems don't become strong enough to fight off asthma and other conditions.

- **Early allergen exposure:** Frequent contact with possible allergens and irritants may increase your risk for developing asthma.

Factors that triggers asthma

- Smoking
- Infections like cold
- Allergens such as food, pollens, dust mites and pet dander
- Exercise
- Air pollution and toxins
- Emotional stress and anxiety
- Weather, especially extreme changes in temperature.
- Drugs (such as aspirin, NSAID and beta blockers)
- Emotional stress and anxiety
- Singing, laughing or crying
- Perfumes and fragrances
- Acid reflux

Allergens are the most causative factor for 50 to 70% for adults in asthma. In children under 3 years of age, viral infections (respiratory syncytial virus) are the most common trigger. After 3 years of age, the allergies also begin to play an increasing role as a trigger. After 20 years of age, occupational exposure to any toxic substances and allergens also can be important triggers

Dietary deficiency of antioxidants may predispose to development of asthma from childhood days [42].

Types of bronchial asthma

As many different factors come together to cause asthma, there are many different types of the disease, separated by age and severity.

Adult and children share the same triggers for symptoms that set off an allergic response in the airways, including airbone pollutant, mold, mildew and cigarette smoke.

Types

- Childhood asthma
- Adult-onset asthma
- Occupational asthma
- Difficult to control and severe asthma
- Seasonal asthma

Childhood asthma

Children are more likely to have an intermittent form of asthma that presents in severe attacks.

Some children might experience daily symptoms, but the common characteristic among children with asthma is a heightened sensitivity to substances that cause allergy.

The Centers for Disease Control and prevention (CDC) advice that children experience more emergency visits and admissions for asthma than adults.

Adult-onset asthma

Asthma in adults is often persistent and requires the daily management of flare-up and preventing symptoms. Asthma can begin at any age.

Allergies lead to at least 30% of adult presentations of asthma. Obesity is a strong risk factor for adult-onset asthma and women are more likely to develop the condition after the age of 20 years.

People over 65 years of age make up a large number of deaths from asthma.

Occupational asthma

This is a type of asthma that occurs as a direct result of a job or profession. Symptoms will apparent after attending a particular workplace. Industries with regular association to occupational asthma include baking, laboratory work or manufacturing. Other symptoms might include a runny nose and red eyes

Difficult to control and severe asthma

These types involve consistent, debilitating asthma symptoms and breathing difficulties. Around 12 % percent of people with asthma have difficult to control or severe asthma.

Seasonal asthma

This type occurs in response to allergens that are only in the surrounding environment at certain times of year, such as cold air in the winter or pollen during hay fever season.

People still have asthma for the rest of the year but do not experience symptoms.

Pathology

- Inhaled allergens stimulate sensory nerve endings called irritant receptors lying below the airway epithelium.
- Stimulation of these irritant receptors causes parasympathetic nerves to release acetylcholine (ACh). When acetylcholine binds to M3 muscarinic receptors on airway smooth muscle, a series of events is initiated which results in an increase in intracellular calcium and smooth muscle contraction (broncho constriction or bronchospasm).
- Some inflammatory mediators such as histamine can also increase intracellular calcium and cause bronchospasm.
- Inflammation of the airways is brought on the several factors like eosinophil, T-lymphocytes (CD4+), macrophages, and mast cells infiltrate the bronchial wall.
- The epithelium is vacuolated and the ciliated cells desquamate. Several cellular factors play their roles in the inflammatory process.
- Neuropeptides such as bradykinins, substance P and neurotension are lead to broncho constriction and excessive secretion of mucous.
- Mast cells initiate the response on exposure to allergens, excessive osmatic changes and variations in temperature.
- Macrophages produce cytokines, which are either broncho constrictor or bronchodilator. Presence of eosinophil in the inflammatory exudate is characterics of asthma.
- Eosinophils are derived from bloodstream. Major basic proteins and cationic proteins of eosinophil lead to destruction of mucosal surface.
- T-lymphocytes, especially CD4+ produce cytokines IL-3,IL-4, IL-5 and GM-CSF which modify the inflammation.

- TNF which is an inflammatory cytokine is expressed in greater amounts by mast cells. The bronco-alveolar secretions contain higher levels of TNF.
- Possibly platelet derived humeral factors also modify the inflammation.
- The main chemical transmitters, which alter the airways, are histamine, prostaglandin and leukotriene. These lead to contraction of bronchial muscle, increase in vascular permeability and excessive secretion of abnormal mucous. Airway inflammation persists for several years. Its severity correlates severity of asthma. Hyper responsiveness of the inflamed airways is aggravated by autonomic and neural mechanisms.
- The final result is obstruction of the small and medium sized airways brought about by mucosal oedema, tenacious mucous and broncho constriction. (43)

Classification

Bronchial asthma classified into 2 groups

1. Extrinsic asthma (Atopic)
2. Intrinsic asthma (Cryptogenic)

Extrinsic

- IgE was raised at least 70%
- Atopic subjects
- Onset was early (10-15 years)
- Intermittent in nature
- Family history of atrophy

Intrinsic

- IgE was normal or low
- Usually Non-atopic subjects
- Onset in middle age (30years)
- Constant in nature
- Family history of asthma.(44)

Clinical Features

Asthma classically displays a diurnal pattern, with symptoms and lung function being worse in early morning.

Typical symptoms are

- Recurrent episodes of wheeze
- Chest tightness
- Breathlessness
- Sometimes Cough

Cough may be a dominant symptom in some asthmatic patients and the lack of wheeze or breathlessness may lead to a delay in reaching the diagnosis of called "cough variant asthma".

The classical aspirin-sensitive patient is female and presents in middle age with asthma, rhino-sinusitis and nasal polyps. Aspirin sensitive patients may also report symptoms following alcohol (white wine) and foods containing salicylates.

Diagnosis

- Diagnosis of bronchial asthma is clinical. The history of sudden attack of paroxysmal dyspnoea, cough and auscultator hallmark of expiratory wheeze heard all over the chest are diagnostic.
- Long duration of complaints history of allergy and positive family history are other helpful clinical points.
- Objective assessment of the severity of airways obstruction and response to broncho dilator therapy can be made by use of bedside peak flow meter.
- Respiratory function tests reveal gross reduction in FEV1, FEV1/FVC ratio and PEFR and increase in the time taken for forced expiration.
- It is important to assess the severity of airways obstruction. Confirmation of the diagnosis of asthma is usually achieved by serial PEFR monitoring. PEFR in the majority of cases shows a diurnal variation of more than 15 % and improvement with therapy.
- When it is necessary to investigate for provocative factors bronchial challenge testing or BPT may be desirable.

Clinical features which indicate severe ventilator impairment are

1. Inability to narrate history continuously or severe distress even on mild exertion
2. Cyanosis, flapping tremors
3. Mental confusion
4. Respiratory rate above 25/min
5. Heart rate persistently above 110/min
6. Inspiratory fall in blood pressure exceeds 16 mm Hg
7. PEFR less than 40 % of predicted value
8. Feeble breath sounds

Differential diagnosis

- Chronic bronchitis
- Cardiac failure
- Pulmonary embolism
- Pulmonary eosinophilia
- Metabolic acidosis
- Emphysema
- Foreign body aspiration.[45]

Management of asthma

In addition to using maintenance medications, their are some steps to make healthier and reduce your risk for asthma attacks. These include:

- **Eating a healthy diet:**

  Eating a healthy, balanced diet can improve overall health, which may reduce the risks for asthma attacks. In that same vein, research suggests that eliminating processed foods may cut down on the risk of asthma attack.

- **Quitting smoking:**

  Irritants such as cigarette smoke can trigger asthma and it also put yourself at greater risk for COPD.

- **Exercising regularly:**

  Activity can trigger an asthma attack, but regular exercise may actually reduce the risk of breathing problems. Aerobic activity can strengthen the lungs and helps to breath better.

- **Managing stress:**

  Stess can be a trigger for asthma symptoms, Stress can also make stopping an asthma attack more difficult. Find healthy ways to reduce the stress and anxiety.

3.5. PHARMACEUTICAL REVIEW

CHOORANAM

Definition

Chooranam are fine dry powder of drugs. The term *"Chooranam"* may be applied to the powder of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity.

Method of preparation

Equipment required

- The drug enumerated in the recipe in clean and dried state.
- A mortar and pestle.
- A fine sieve or fine cloth of close mesh.

Process of preparation:

- The drugs which are to be used in the preparation should be taken from recently collected material. Drugs which are aged by prolonged storage or changed in colour, taste and scent and those that are insects infected or attacked by fungi should be positively rejected.
- The raw drugs are chopped and dried in sun or shade completely and the drugs are pounded in stone morter then sieved through a fine mesh.
- If the raw drugs are pounded together it will not yield a beneficial result.
- Some substances before pounding are roasted. The substances to be roasted are kept in an earthen ware and under minimal heat separately. In case if the different substances to be roasted together, some drugs are roasted earlier and some are burnt under the same heat. So they should not be roasted together.

- In general, the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic, should be removed from the drugs by close inspection.
- The *Chooranam* should be as fine as to be called amorphous and should be never damp. The fitness of the sieve should be 100 mesh or still finer.

Purification of the prepared *Chooranam*

"தானென்ற சூரணத்தின் சுத்திக்கேளு
தப்பாதே சரக்கெல்லாஞ் சூரணித்து
நானென்ற வாவின் பாலாற் பிசைந்து
நலமான சட்டியிலே பாலைவிட்டு
வானென்ற சுத்தசலம் பாதிவிட்டு
வளமாக மேற்சீலை கோடு கட்டிப்
பானென்ற சூரணத்தைப் பிட்டுபோல் வைத்து
பதறாதே வெந்தெடுக்கச் சித்தியமே."

- அகத்தியர் வைத்திய இரத்தினச் சுருக்கம்

The prepared *Chooranam* is mixed with the milk in a pot half a quantity milk and half a quantity water is taken.

The mouth of the pot is covered with a thin cloth material. Above this cloth the mixed *Chooranam* is placed. The pot is covered with lid and heated.

"ஆமப்பா ரவியுலர்த்திப் பொடிதான் செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து
நாமப்பா கொண்டு வர தோஷம் போச்சு
நன்றாகச் சுத்தி செய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லா தப்பா
தளமான வியாதி யெல்லாம் பாரிக்கும் பார்
வேமப்பா சுத்தி செய்து கொண்டாயானால்
வெகுசுறுக்காய் தீருமா வியாதி கேளு".

- அகத்தியர் வைத்திய இரத்தினச் சுருக்கம்[46]

Then the *Chooranam* is dried in the sunlight and powdered. Equal amount of sugar is added and taken internally. Many types of disease get cured. If the drug is taken without purification the diseases does not cure. If taken after purification the disease gets cured easily.

Storage

The prepared *Chooranam* should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly stoppered glass, polythene or tin containers or cellophane bags and sealed. These bags should in turn be enclosed in card board boxes.

The *Chooranam* to facilitate easy handling and to assure exact dosage of administration could be pressed into tablets with the addition of a suitable binder. These tablets could be packed in bottles or tubes made either of glass or packed in strip of metal foil or plastic sheets.

Shelf life of medicines

Medicines can be classified into internal and external medicines .They are each in 32 types. Chooranam comes under the category of internal medicines. The shelf life of medicines indicates the potency of medicines. The medicine even though seems to be fresh is not efficacious after sometime. So the medicines should not use after certain period.

As per siddha literature *Agamarunthu padal* in *Gunapadam Thathu-seevam* text

உயர்சூர ணம்பிட்டு வடகம் வெண் ணெய்நான்கி

னுயிர்மூன்று திங்களாகும்[47]

From the above quote, the shelf life of *Chooranam* (powder) is 3 months. But According to AYUSH guidelines the shelf life of *Chooranam* (powder) is 1 year [48]. The *Chooranam* is said to retain its potency for three months and then gradually deteriorate. However, if properly packed and stored they keep good for a year as per AYUSH guidelines.

ANALYTICAL SPECIFICATIONS OF CURNA/ CHOORNAM

S.NO	TESTS
1.	Description Macroscopic, Microscopic
2.	Loss on drying at 105^0c
3.	Total - ash
4.	Acid – insoluble ash
5.	Water- soluble extractive
6.	Alcohol- soluble extractive
7.	Particle size (80-100 mesh for churna; 40-60 mesh for kvatha churna)
8.	Identifications, TLC/HPLC- with marker (wherever possible)
9.	Test for heavy/ Toxic metals Lead Cadmium Mercury Arsenic
10.	Microbial contamination Total bacterial count Total fungal count
11.	Test for specific pathogen E. coli Salmonella spp. S. aureus Pseudomonus aeruginosa
12.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
13.	Test for Aflatoxins(B1, B2, G1, G2)

3.6. PHARMACOLOGICAL REVIEW

A. REVIEW OF DRUG (Modern Medicine)

BRONCHODILATOR DRUGS USED

- A bronchodilator is a substance that dilates the bronchi and bronchioles, decreasing resistance in the respiratory airway and increasing airflow to the lungs.
- Bronchodilators may be endogenous (originating naturally within the body), or they may be medications administered for the treatment of breathing difficulties.
- They are most useful in obstructive lung disease of which asthma and COPD are the most common conditions.

Types of bronchodilator drugs

Bronchodilators are either short-acting or long acting. Short-acting bronchodilators are predominantly used as preventers.

There are three types of bronchodilators namely

1. β2-agonists (short and long-acting)
2. Anticholinergic (short and long- acting)
3. Theophylline (long-acting)

1.β2-agonists

(a) Short-acting β2-agonists

- This medication is providing quick or "rescue" relief from acute bronchoconstriction.
- These medications usually take effect within 20 minutes or less, and can last from 4 to 6 hours.
- These inhaled medications are best for treating sudden and severe or new asthma symptoms.
- Taken 15 to 20 minutes ahead of time, these medications can also prevent asthma symptoms triggered by exercise or exposure to cold air.

Examples:

Salbutamol, Levosalbutamol, Pributerol, Terbutaline, Epinephrine, Ephedrine.

(b)Long –acting β2- agonists

- These are long term medications taken routinely in order to control and prevent broncho constriction.
- These medications may take longer to begin working, but relief airway construction for up to 12 hours.
- Commonly taken twice a day with an anti-inflammatory medication, they maintain open airways and prevent asthma symptoms, particularly at night.

Examples:

Salmeterol, Fenoterol, Pirbuterol, Clebuterol, Formoterol, Bambuterol, Indacaterol [49]

(2)Anticholinergics

- Anti cholinergics or Anti muscarinic drugs relax the smooth muscles but response is slower than Beta-2 agonist.
- Some examples of anticholinergics are Tiotropium bromide and Ipratropium bromide.
- Tiotropium bromide is long acting, a single inhalation can have effect lasting for 24 hours. It reduces the frequency and severity of episodes.
- Ipratropium bromide is short acting, given by inhalation which has effect for 4-6 hours.

(3)**Theophylline**

- Theophylline is long acting bronchodilator that prevents asthma episodes.
- Available in oral and injectable form.
- It is prescribed in severe cases of Asthma or those that are difficult to control.
- Blood tests are required to monitor therapy and to indicate when dosage adjustment is necessary [50].

MECHANISM OF ACTION OF BRONCHODILATOR DRUGS

- Beta-2 agonist drugs bind to beta-2 receptors on airway smooth muscle relaxation. When airway smooth muscle relaxes, the diameter of the air passages is enlarged.
- Bronchodilator drugs blocks the action of phosphordiesterases and prevents the breakdown of cAMP to 5-AMP. This also has the effect to relaxing smooth muscle and allowing the airways to dilate.
- The bronchoconstriction effects of acetylcholine can be blocked by muscarinic antagonists. Muscarinic antagonists bind to muscarinic receptors and prevent acetylcholine from binding.
- Bronchodilator can also be achieved by alpha-2 agonist drugs that bind to alpha 2 receptors on parasympathetic nerves and prevent acetylcholine from being released[(51)].

B. PHARMACOLOGICAL STUDY IN ANIMAL MODELS

BRONCHODILATOR ACTIVITY

In vitro methods

Spasmolytic activity on guinea pigs isolated tracheal chain

The isolated tracheal chain of guinea pigs can be used for testing compounds which inhibit bronchospasm. It detects sympathomimetic, H_1-histamine receptor antagonist properties of test drug.

Methodology

Guinea pig of either sex weighing between 300-500 g are sacrificed using ether anaesthesia. The entire trachea is dissected out and cut into individual rings. Twelve to fifteenrings are tied together with silk threads and mounted in the organ bath containing Krebs-Henseleit solution and maintained at 37^{o}C, under a tension of 0.5 g and gassed with carbon. Isometric contractions are recovered via a strain gauge transducer on a polygraph. Forty five minutes are allowed for equilibration before the addition of the spasmogen. The following spasmogens used Histamine, Carbachol, LTC_4 or LTD_4. It takes about 10-12 min for reaching the contraction to a maximum. At this stage, standard

and test drugs are administered. The bronchial response is allowed to plateau and recorded. The tissue is rinsed thoroughly and the control contractions are induced again by adding spasmogen. The percent of inhibition of spasmogen induced contractions is calculated. From dose response curve ED_{50} is calculated. (52)

Isolated Frog Rectus Abdominis Muscle Preparation

A frog is pithed and laid out on frog dissection board. The skin of the anterior abdominal wall is cut by a midline incision which is extended laterally up to the anterior aspects of the limbs. This exposes the flat whitish muscle of the anterior abdominal wall from their pubic origin to their sternal insertion. The two recti are removed and placed in frog ringer solution in a shallow dish. They are carefully cleaned and one of them is trimmed to the desired size and mounted in an organ bath of 5ml capacity, at room temperature and aerated with oxygen. For recording purposes, an isotonic lever with a sideways writing point is used tangential to the smoked drum, balanced for a tension of 2.5gm with an extra load of 1gm on the long arm. A standard solution of Acetylcholine is added to the bath and a slow contraction is recorded on the slow moving drum for exactly 90sec. The drum is stopped and the bath fluid is replaced by fresh Frog-Ringer. An extra 1gm load is used to extend the muscle to its original length. (53).

In vivo methods

Histamine induced bronchospasm in guinea pig

Guinea pigs subjected to inhibition of aerosols containing histamine or other bronchospasm inducing agents, exhibits the symptoms of asphyxiating convulsions resembling acute attack of bronchial asthma. These challenging agents are administered in the form of aerosols through a nebulizer to individual guinea pigs placed in a histamine chamber. The initial symptoms are increased frequency of breathing, forced breathing and finally asphyxiating convulsions. The occurrence of these symptoms can be delayed by antagonistic drugs and bronchodilators. Pre-convulsion time is noted as the end point.

Methodology

Male guinea pigs weighing around 400 grams are used in groups of 8-10 animals. The animals are treated with test / standard drugs orally or subcutaneously. The animals are then placed in the standard Histamine chamber, 30 minutes after the administration of drug and exposed to an aerosol of 0.1 % solution of histamine dihydrochloride through a nebulizer. Time required for the onset of asphyxiating convulsions is recorded. The animal is immediately withdrawn from the inhalation box and placed in a well-ventilated area for revival from the convulsions. This method has been further improvised using an ultra-sound nebulizer which provides the steady exposure to histamine solution at a pre-determined rate. Percentage increase of pre-convulsion time is calculated from the average values of treated and control groups of guinea pigs. ED_{50} values denoting 50% increase in the pre-convulsion time can also be calculated. Histamine aerosol exposure is a very commonly used and dependable method for screening the bronchodilator activity of novel compound.[54]

Egg albumin induced anaphylaxis in guinea pig

Guinea pig was sensitized by two intra peritoneal injections of 0.5 ml and 10% w/v solution of egg albumin at a 48 hours interval. After sensitization, the animals were divided into two groups. Animals of group I received 0.5% CMC and serve as control group. Animals of group II received ethanolic extract trial drug (500 mg/kg. once daily) dissolved in distilled water for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms and mortality [55].

ANTI- HISTAMINE ACTIVITY

Effects of diphenhydramine in experimentally produced asthma in guinea pigs

Aim

To demonstrate the antagonistic effects of diphenhydramine against histamine induced bronchospasm in the guinea pig.

Principle

difficulty in breathing and convulsion. These effects of histamine are mediated through the action of histamine on H_1 receptors. Diphenhydramine is a H_1 receptors blocker. Therefore, diphenhydramine prevents the bronchospasm induced by histamine. Guinea pig is very sensitive to histamine. When guinea pig is exposed to histamine vapour it exhibits bronchospasm.

Equipments and other materials required

Histometer, stop watch, disposable needle and syringes.

Animal : Guinea pigs

Drug solutions required

- Normal saline
- Diphenhydramine 5 mg/ml
- Histamine diphosphate 30µg/ml

Procedure

Select 4 guinea pigs having body weight between 250-350 grams. Fast the guinea pigs for 12 hours before the experiment. Divide the guinea pigs into 2 groups of 2 animals each. Weigh the guinea pigs in each group and mark them for identification. Administer the drug solutions as Group I Normal saline 1 ml/kg, Group I Diphenhydramine 5 mg/kg. After one hour place each guinea pig in histamine chamber and replace the cover. With the help of compressor, spray a finely atomized mist of histamine diphosphate from nebulizer in both comparments. Using a stop watch, record the time of histamine administration. Observe the signs of respiratory distress and the animal falling on its side and record the observations [56].

Isolated Guinea Pig Ileum

Overnight fasted guinea pigs of either sex weighing 400-600gram were sacrificed using cervical dislocation method. The lower most 10cm of ileum was removed from the abdomen and placed in a shallow dish containing warm Triode solution. Ileum lumen was cleaned by passing through warm 0.9% saline and then segments about one inch in

length, were made. The mesenteric attachment and blood etc. were carefully cleaned and tissues was mounted in a thermostatically controlled Dale's organ bath containing 20ml Triode's solution under basal tension of 500mg. the composition of solution in was Nacl, 137; $Cacl_2$, 1.8; Kcl, 2.7; glucose, 5.55; $NaHco_3$, 11.9; $Mgcl_2$, 1; $NaH2Po_4$, 0.4. The solution was continuously bubbled with air. The responses to drug were recorded on a student physiography using isotonic transducer, which exerted a basal tension equivalent to 500mg load tissue. The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min. Increasing concentration of histamine were added to the bath and the control cumulative concentration-response curve was constructed [(57)].

C. REVIEW OF SIDDHA DRUGS

List of some Siddha drugs used in Bronchial Asthma

- *Swasakudoori Mathirai*[(58)]
- *Vasantha Kushmagaram*[(58A)]
- *Gowri Chinthamani Chendhooram*[(58B)]
- *Thalisadi Chooranam*[(59)]
- *Pavala Parpam*[(58C)]
- *Kashthoori Karuppu*[(58D)]
- *Adathodai Chooranam*[(60A)]
- *Swasakasa Matthirai*[(60B)]
- *Kodasoori Kuligai*[(58E)]
- *Milagu Chooranam*[(61)]
- *Thalaga karuppu*[(58F)]
- *Karpooradhi Chooranam*[(62)]
- *Swasakrudhum*[(62A)]

- *Sivanar Amirtham* (58G)
- *Thooduvalai nei*(58H)
- *Mahathalisapatthira Chooranam*(63)
- *Arakku thailam*(58I)
- *Soombu theneer*(58J)
- *Adhatodai kudineer*(58K)
- *Nochi Thailam*(58L)

D. LATERAL RESEARCH

Solanum xanthocarpum:

Hypoglycemic activity of *Solanum xanthocarpum* (64)

The aqueous extract showed significant hypoglycemic effect in both normal and streptozotocin induced diabetic rate at dose of 100 and 200mg/kg. The avtivity showed by aqueous extract was comparable to that of standard oral hypoglycemic agent glibenclamide. The experimental result indicated that it exhibited a potent blood glucose lowering property both in normal and streptozotocin induced diabetic rats. The LD_{50} of the extract was found tobe high indicating high margin of safety.

Antifilarial activity of *Solanum xanthocarpum*

Latin Mohan et al. reported the larvicidal potential of crude extracts of *Solanum xanthocarpum* and suggested its suitability as an ecofriendly, effective larvicide in the management of mosquito populations and in limiting the outbreak of various vector borne epidemics.

Hepatoprotective activity of *Solanum xanthocarpum* (65)

In Chandana VR et al. investigation, *Solanum xanthocarpum* extracts was evaluated for hapatoprotective activity using CCL_4 induced hepatotoxicity in rats. The hepatotoxicity induced by CCL_4 is due treated with *Solanum xanthocarpum* extracts showed significant increased in the level of enzyme which indicates the antioxidant activity of *Solanum xanthocarpum*. Jigrineis polypharmaceutical herbal formulation

containing aqueous extracts of 14 medicinal plants including *Solanum xanthocarpum* and used for liver ailments.

Anti-Fertility activity of *Solanum xanthocarpum* (66)

Solasodine, an alkaloid of *solanum xanthocarpum* possesses antispermatogenic activity. In Dixit VP 1980 study, chronic administration of solasodine (20mg/ kg each other day oral for 60 days) rendered male rats and dogs infertile. Mating test showed 87% infertility in rats, this returned to normal after 60 days cessation of drug feeding. Solasodine is well tolerated and inhibits spermatogenesis and sperm motility. No significant change was noticed in the weight of testes and accessory sex organs. The RNA, protein, salicylic acid and glycogen contents of the test were reduced significantly, serum proteins, triglycerides, Serum enzymes (GOT/GPT / Alkaline phosphatase) nonesterified fatty acids levels were in normal range. Solasodine is estrogen free but inhibits testosterone release from dispersed mouse Leydig cells (200 Um release). Solasodine can be developed as male pill of plant origin.

Piper longum:

Immunomodulatory and Anti-tumor activity of *Piper longum*

Alcoholic extracts of the fruits was 100% toxic at a concentration of 500 micro/ml to Dalton's lymphoma ascites (DLA) cells and 250 micro/ml to Ehrlich ascites carcinoma (EAC) cells. Piperin was found to be cytotoxic towards DLA and EAC cells at a concentration of 250 micro/ml. Alcoholic extracts and piperin was also found to produce cytotoxicity towards L929 cells in culture at a concentration of 100 and 50 mico/ml, respectively (67)

Anti-hyperglycemic activity of *Piper longum*

The aqueous and methanolic extracts of *piper longum* root produced significant anti-hyperglycemic activity at a dosage of 200mg/kg b.w in diabetic treated rats.

Antimicrobial activity of *piper longum*

Various extracts of *Piper longum* were prepared and evaluated against bacterial pathogens, such as *Salmonella albus typhi*, *Pseudomonas aeruginosa*,

Escherichia coli and *Bacillus megaterium* and one fungus, *Aspergillus.niger*. Compared to streptomycin all the extracts exhibited a good antibacterial activity. The isolated constituents and n-hexane extract were found to show varying degree of antibacterial activity against all the tested bacteria. However the aqueous extract did not show antibacterial activity against the tested bacteria [68].

Piper nigrum:

Anti-inflammatory activity and Anti-Rheumatic activity of *piper nigrum*

Piperin has anti-rheumatic effects in animal models and anti-inflammatory effects on IL1β-stimulated FLSs. Piperin also inhibited the activation of the transcription factor AP-1, but not NFκB, in our system.

Antibacterial activity of *Piper nigrum*

The acetone extract of black pepper displayed excellent inhibition on the growth of Gram positive bacteria. Staphylococcus was susceptible followed by Bacillus and Streptococcus. The MIC values are 125, 250 and 500 ppm, respectively. Among the Gram negative bacteria Pseudomonas was more susceptible to black pepper followed by E.coli, klebsiella and salmonella [69].

Zingiber officinale:

Antimicrobial activity of *Zingiber officinale*

Ingenol and [6]- shogaol, isolated from ginger rhizome, demonstrated antiviral activity.32 [10]-gingerol has been reported as active inhibitor of *Micobacterium avium* and *Micobacterium tuberculosis* in vitro. Gingerol and related compounds have been investigated for antimicrobial activities. [6]- gingerol and [12]- gingerol, isolated from ginger rhizome, demonstrated antibacterial activity against periodonatal bacteria.

Antinociceptive activity of *Zingiber officinale.*

[6]-shogaol has produced anti-nociception and inhibited the release of substance p in rats, seemingly via the same receptor to which capsaicin binds. However, it was observed to be 100 times less potent and to elicit half the maximal effect of capsicin.

Toddalia asiatica

Antibacterial activity of *Toddalia asiatica*

In vitro antibacterial activities of different plant extracts were tested against various clinically important microbial pathogens of Enterobacter faecalis, proteus vulgarisand serratia marcescens procured from the department of microbiology, RVS College of arts and science, Coimbatore and were maintained on nutrient agar slants. 0.2ml of overnight grown cultures of each organism was dispensed into 20ml of sterile nutrient broth and incubated for 3-5 hrs at 37^0 C to standardize the culture to 10^6 CFU/ml8. 10µ plant extract (40mg/0.1ml) was soaked by sterile filter paper discs were impregnated with plant extract placed on the surface of the medium and incubated at 37^0C for 24 hrs. The assessment of antibacterial activity was measured around the disc [70].

MATERIALS AND METHODS

4. MATERIALS AND METHODS

In this dissertation "*Kandankathari Chooranam*" was taken as a trail drug from the Siddha literature "*Akathiyar Attvanai Vagadam*" - First Edition, page no: 63, Published by Dr.S.Arangarasan, Saraswathi Mahal Library,Thanjavur.

Ingredients of the drugs:

1. Kandankathari Root *(Solanum xanthocarpum)* - 43gm
2. Araikeerai (*Amaranthus dubius*) - 85gm
3. Chukku *(Zingiber officinale)* - 255gm
4. Thippili *(Piper longum)* - 255gm
5. Milagu (*Piper nigrum)* - 255gm
6. Milagaranai pattai (*Toddalia asiatica*) - 85gm

Associated drugs

7. Sugar (Saccharum officinarum) - QS
8. Ghee - QS

Collection of the raw material

All the raw materials were bought from Ramasamy chetty country drug store, Parry's corner, Chennai.

Identification and Authentication of the drug

All the raw materials were identified and authenticated by the experts of Gunapadam. Government Siddha Medical College, Arumbakkam, Chennai-106. The specimen sample of all the raw drugs have been preserved in PG Gunapadam department individually for future reference.

4.1. PREPARATION OF THE DRUG

Purification of the drug

Purification Process for all the drugs mentioned here were done as per various classical Siddha literatures [71].

Kandankathari Ver : The root was cleaned with white cloth. Finally washed and dried

Araikeerai : Leave were washed in running water and finally dried.

Chukku : Skin of dried ginger was peeled off.

Thippili : Soked in 250 ml of lemon juice and it was dried.

Milagu : Soked in 250ml of buttermilk for 3 hours and Dried.

Milakaranai vaerpattai : Dust and odd materials were removed.

Preparation of the drug

Procedure

The purified ingredients were Pounded separately until it turns to a fine powder. Then the powder was sieved through a white cloth and all the powders were mixed well. The *Chooranam* was slightly roasted by adding ghee and sugar. The prepared *Chooranam* was kept in an air tight container and was lableled as *"**Kandankathari Chooranam"*** (KKC).

Purification of the *Chooranam*:

***Pittaviyal murai* (Milk Steaming process):**

The ***Kandankathari Chooranam*** was purified by *Pittaviyal* method (milk steam cooking) as per Siddha classical literature. A mud pot was taken and it was quarter filled by milk and quarter filled by water. The mouth of the pot was sealed by a cloth. This *Chooranam* was then placed over the cloth and the pot was covered with lid and heated. The same drug was later dried, powdered then sieved again. It was used for the further study [73].

Preservation of the drug

The prepared test drug was stored in a clean, air tight glass container.

Administration of the drug

Form of the medicine	:	*Chooranam* (powder)
Route of administration	:	Enteral
Dose	:	Pakkalavu (6.022gm)
Vehicle	:	Hot water
Indications	:	Swasa kasam (Bronchial Asthma) Irumal (Cough)

INGREDIENTS OF KANDANKATHARI CHOORANAM:

Fig no 1.1: ***Kandankathari*** **(*Solanum xandhocarpum*)**

Fig no 1.2 : ***Araikeerai*** **(*Amaranthus tristis*)**

Fig no 1.3: ***Chukku*** ***(Zingiber officinale)***

Fig no 1.4: ***Thippili*** ***(Piper longum)***

Fig.1.5: ***Milagu***
(Piper nigrum)

Fig.1.6: ***Milagaranai vaerpattai***
(Toddalia asiatica)

Fig.1.7. ***Ghee***

Fig.1.8. ***Sugar***
(Saccharum officinarum)

Fig. 1.9. Preparation of the *Kandankathari Chooranam*

Fig.2. *Kandankathari Chooranam*

4.2. STANDARDIZATION OF THE DRUG

Standardization of the drug brings the validation of a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and

phytochemical properties and also to assess the active principles and elements present in the drug.

Method of standardization:

Techniques Involved In Standardization of Compound Drugs:

- Macroscopic Methods
- Microscopic Methods
- Physical Methods
- Chemical Methods
- Biological Methods

4.2.1. ORGANOLEPTIC CHARACTER:

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odor, taste, texture etc.

Colour:

A sample of *Chooranam* were taken in watch glasses and placed against white back ground in white tube light. The *Chooranam* were observed for its color by naked eye.

Odour:

Chooranam were smelled, the time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste:

A sample of *Chooranam* was tasted and the taste was reported.

Size:

The *Chooranam* was completely sieved through mesh size 88.

4.2.2 PHYSICOCHEMICAL ANALYSIS:[(74)]

Physicochemical- studies of the trial drug have been done according to WHO Guidelines. Physico chemical studies like total ash, water soluble ash, acid Insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, The Tamilnadu Dr. MGR Medical University, Chennai.

1. Solubility Test:

A pinch of sample (*KKC*) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Chloroform and Ethyl alcohol the results are observed individually.

2. pH value:

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the ***Kandankathari Chooranam*** was written in results column.

3. Loss on Drying:

An accurately weighed 1gram of ***Kandankathari Chooranam*** was taken in a tarred glass bottle. The crude drug was heated 105^0 c for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

4. Determination of total Ash:

Weighed accurately 2grams of ***Kandankathari Chooranam*** was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

5. Determination of acid insoluble ash:

Ash above obtained was boiled 5 minutes with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

6. Determination of water soluble ash:

Total Ash 1gram was boiled for 5 minutes with 25ml water and insoluble matter collected on an ash less filter paper was washed with water and ignited for 15 minutes at a temperature not exceeding 450^0c in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

7. Determination of water soluble extractive:

4grams of air dried drug, Coarsely powered ***Kandankathari Chooranam*** was macerated with 80ml of distilled water in a closed flask for twenty-four hours, shaking

frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 100^0c and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

8. Determination of alcohol soluble extractive:

1gram of air dried drugs; coarsely powdered ***Kandankathari Chooranam*** was macerated with 20ml alcohol in closed flask for 24 hours. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was evaporated in a tarred flat bottom shallow dish, dried at 100^0c and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

4.2.3. PHYTOCHEMICAL ANALYSIS:

The preliminary phytochemical screening test was carried out for each extracts of ***Kandankathiri Chooranam*** as per the standard procedure [74].

1. Detection of Alkaloids:

Extracts were dissolved in dilute hydrochloric acid and filtered.

a) **Dragendroff's test**: Filtrates were treated with Dragendroff's reagent (solution of Potassium with Bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

2. Detection of Carbohydrates:

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

a) **Molisch's test**: To 2ml of a sample extract, two drops of alcoholic solution of alpha naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.

3. Detection of Glycosides:

Extracts were hydrolyzed with dilute HCl and then subjected to the test of glycosides.

a) **Cardiac glycoside (keller-killiani test)**: Extract was shaken with distilled water (5ml). To this, glacial acetic acid (2ml) containing few drops of ferric chloride

was added followed by H_2SO4 (1ml) along the side of the test tube. The formation of the brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

4. Test for Saponins:

a) **Foam test**: 0.5gm of extract was shaken with 2ml of water if foam produced persists for ten minutes. It indicates the presences of saponins.

5. Detection of phenols:

Ferric Chloride test: Extracts were treated with 3- 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of the phenols.

6. Detection of tannins:

Gelatin test: The extract was dissolved in 5ml of distilled water and 2ml of 1% solution of Gelatin containing 10% NaCl was added to it. White precipitate indicates the presence of phenolic compounds.

7. Detection of flavonoids:

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

8. Detection of Proteins:

a) Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of Aminoacids:

a) Ninhydrin Test: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10. Detection of Diterpenes:

a) Copper acetate test: Extracts was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

11. Gum and Mucilage:

To 1ml of extract, 2.5 ml of absolute alcohol was added and stirred constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

12. Test for Quinones:

Extract was treated with sodium hydroxide, blue or red precipitate indicates the presence of Quinones.

13. Test for Fixed oils and Fats:

a) **Spot Test**: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

HPLC - High Performance Liquid Chromatography (HPLC) [(75)]:

HPLC is a technique in analytical chemistry which is used to separate the components in a mixture, to identify each component and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly different with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. In this study, the detection and quantitation were carried out using 515 HPLC pumps and 2489 UV/Visible detectors of Waters Company while the software used was Empower.

Two methods using different mobile phases were used for chromatographic separation of the research drugs – Method I (binary gradient method of Acetonitrile & 0.1% Phosphoric acid in Water) and Method II (binary gradient method of Methanol 1:25 Acetic acid in Water). Results obtained during Method I have been discussed since better separation of compounds was observed during this analysis. The chromatographic conditions for Method I are as given below:

- Column : Symmetry C18, 5 µm, 4.6x250 mm
- Run Time : 30 minutes
- Injection Volume : 20 µl

- Wavelength (Dual) : 272 nm & 360 nm
- Solvent A : Acetonitrile
- Solvent B : 0.1% Phosphoric acid in water
- Flow rate : 1.0 ml/min.
- Pump Mode : Gradient

4.2.4 BIO-CHEMICAL ANALYSIS [76]

The bio-chemical analysis was done to identify the acid and basic radicals present in the *KKC*.

Preliminary Basic and Acidic radical studies

Preparation of extract

5gm of *KKC* was taken in a 250 ml clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Test for basic radicals

1. Test for Potassium

To a pinch of the *KKC*, 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of *KKC* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium:

To 2ml of *KKC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *KKC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution were added and observed for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the *KKC*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. Test for Iron (Ferrous)

KKC extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *KKC* extract, drops of sodium hydroxide solution was added and Observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *KKC* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of *KKC* extract 2ml of potassium iodide solution was added and observed for the appearance for yellow coloured precipitate**.**

10. Test for Copper

A pinch of *KKC* was made into a paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame and observed for blue colour appearance.

To 2 ml of *KKC* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the *KKC* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the *KKC* extract 2ml of sodium hydroxide solution was added and observed for brown or red precipitate.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the *KKC* extract, 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *KKC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. **Test for Phosphate**

The *KKC* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. Test for Carbonate

The *KKC* extract was treated with conc. Hcl and observed for froth appearance of effervescence.

5. Test for Fluoride & Oxalate:

To 2ml of *KKC* extract 2ml of dil. acetic acid and 2ml of calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate:

To 1 gm of the *KKC* copper turnings was added and again conc. H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

4.2.5. AVAILABILITY OF MICROBIAL LOAD

ANTIMICROBIAL ACTIVITY ([77])

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

MATERIALS REQUIRED

1.Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

1. *Escherichia coli* (ATCC 25922)
2. *Staphylococcus aureus* (ATCC 25923)
3. *Pseudomonas aeroginosa* (ATCC 27853)
4. *Klebsiellapneumoniae*(ATCC 13883)

PROCEDURE

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Escherichia coli, Pseudomonas aeroginosa, Klebsiella pneumonia a*nd *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL and 1000µg/mLwere added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

ANTIFUNGAL ACTIVITY [78]

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

MATERIALS REQUIRED

1) **Potato Dextrose Agar Medium (1 L)**

The medium was prepared by dissolving 39 g of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Clotrimazole (standard antifungal agent, concentration: 10mg / ml)

3. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

- *Aspergillus niger* (ATCC 16404*)*

PROCEDURE

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993).

4.2.6. SOPHISTICATED INSTRUMENTAL ANALYSIS

FTIR - Fourier Transform Infra-red Spectroscopy [79]:

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterise some inorganics. Examples include paints, adhesives, resins, polymers, coatings and drugs. FTIR is an effective analytical instrument for detecting functional groups.

APPLICATIONS:

- Quantitative scans and Qualitative scan
- Solids, liquids, gases
- Organic samples, inorganic samples
- Unknown identification and Impurities
- Screening formulation
- Pharmaceuticals.

Fig no.3.1: FTIR INSTRUMENT

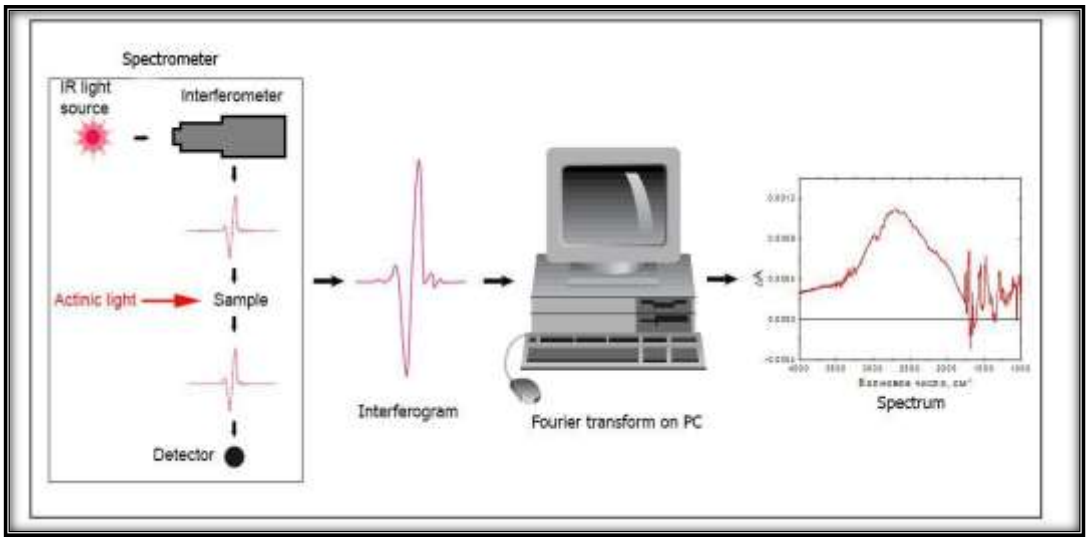


Fig no.3.2: FTIR MECHANISM

Principle:

Spectrophotometric tests are commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures are applicable to substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›) :

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable dye.
- Submit it to the pressure of about 800 mPa (8 tons/cm2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.

- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

XRD (X-RAY POWDER DIFFRACTION) [81]

Definition

X-ray powder diffraction is most widely used for the identification of unknown Crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

Applications

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to,

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)

Characterize thin films samples by:

- Determining lattice mismatch between film and substrate and to inferring stress and strain.
- Determining dislocation density and quality of the film by rocking curve measurements
- Measuring super lattices in multilayered epitaxial structures

- Determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Power Diffraction Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward

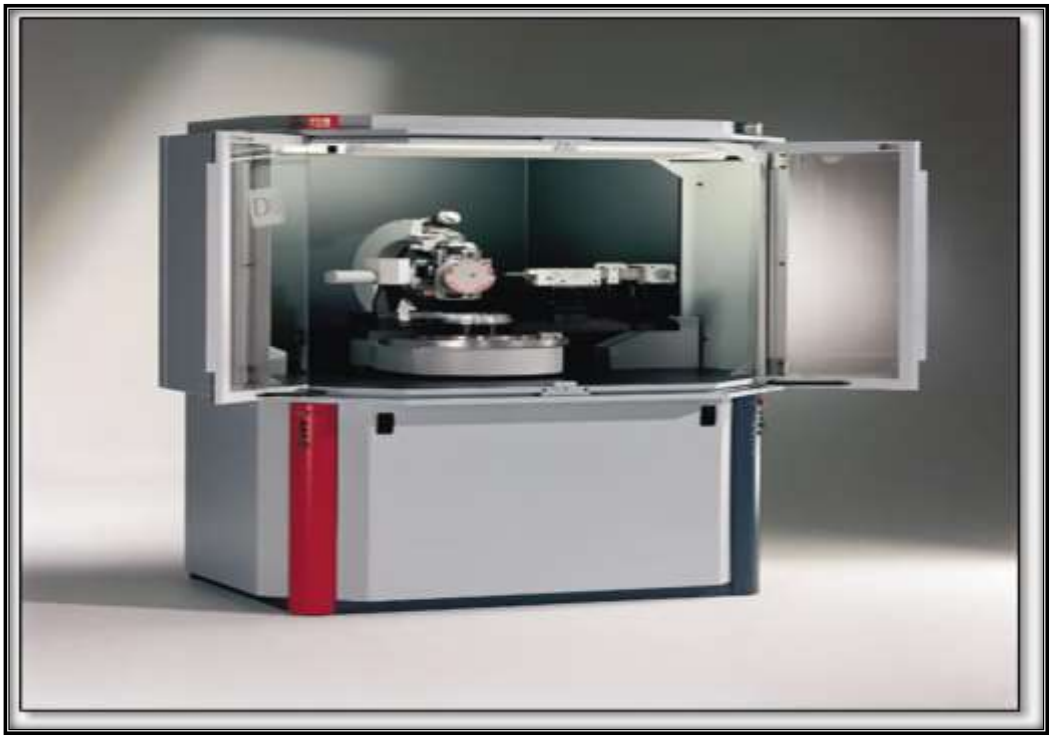


Fig no:3.3: Shows Image XRD Analyser

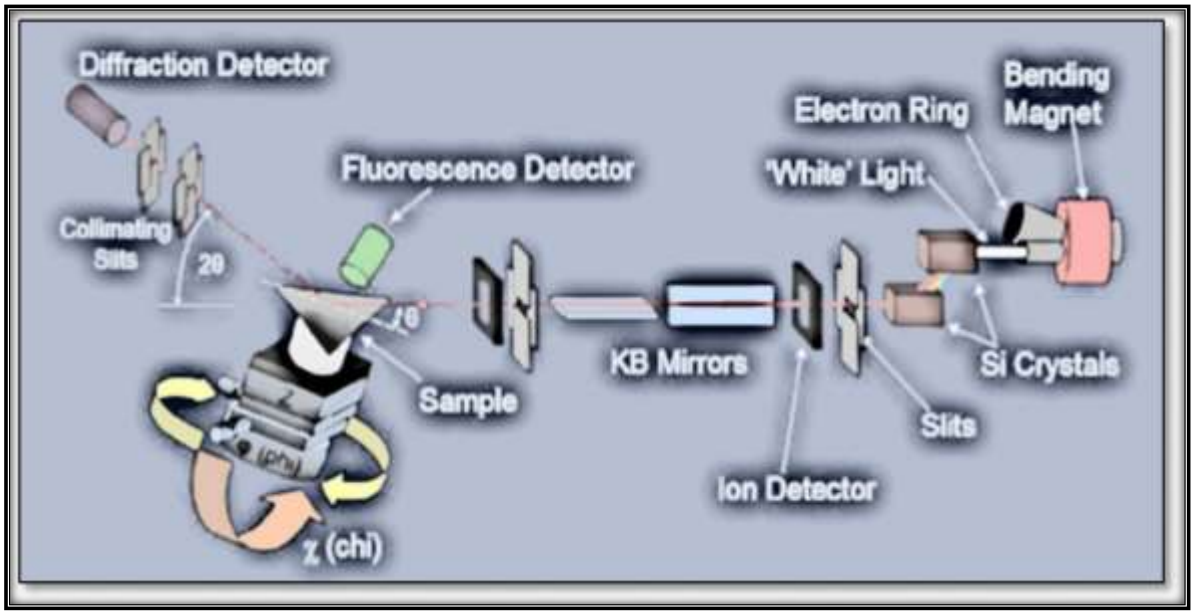


Fig no:3.4: XRD Mechanism

Limitations

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation

Determination of an unknown requires: the material, an instrument for grinding and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
- Powder less than ~10 μm (or 200-mesh) in size is preferred place into a sample holder or onto the sample surface

ICP-OES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY) [82]

Fig: 3.5: ICP-OES INSTRUMENT (Perkin Elmer Optima 5300 DV)

Manufacturer: Perkin Elmer Model

Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP) Principle: An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone (8,000–10,000ºC). The analysts are heated (excited) in different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant (such as the concentration of ferrous iron or Ferric Iron), only total essential concentration is analysed by ICP-OES.

Application

The analysis of major and minor elements in solution KKC.

Objectives

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements Probes the outer electronic structure of atoms.

Mechanism

In plasma emission spectroscopy (OES), a sample solution is presented into the core of Inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values. The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, Chennai-36 using Perkin Elmer Optima 5300 DV. Sample preparation: Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis. 100 mg RSP was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes until the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

The digested sample solution was shifted into plastic containers and labelled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

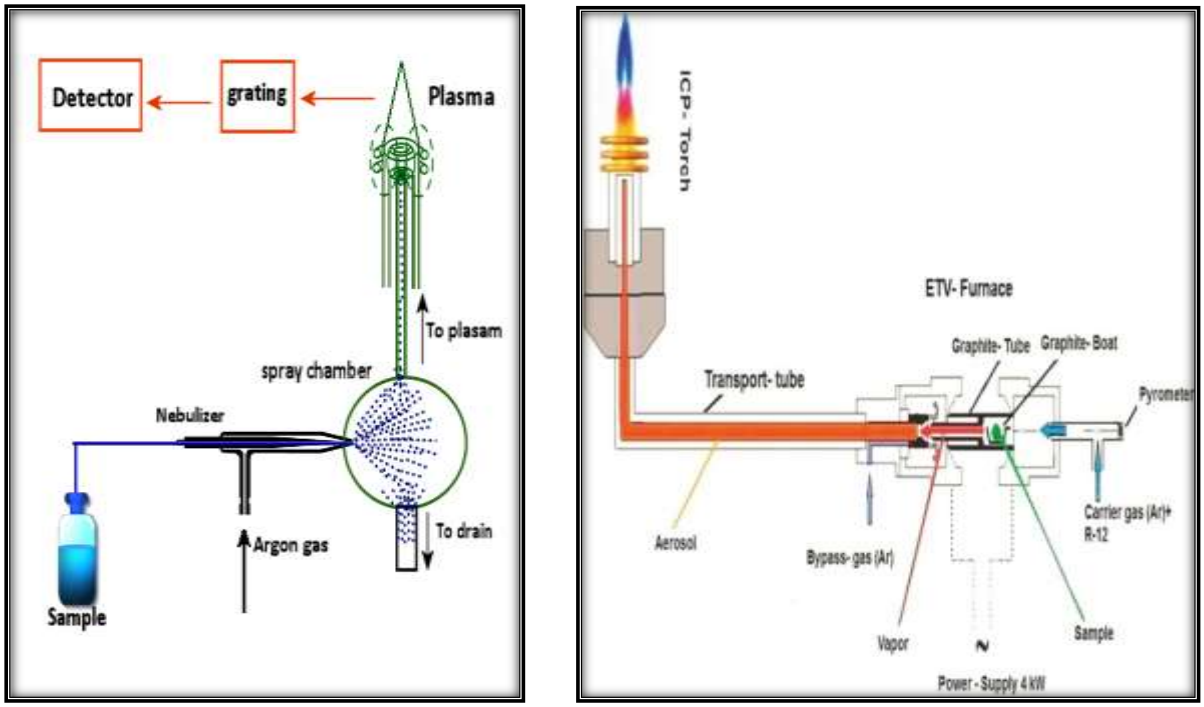


Fig.5.2. ICP-OES MECHANISM

SEM (Scanning Electron Microscope) (83)

DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture), Chemical composition (when used with EDS) Orientation of materials making up the sample.

The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal
- SEM Analysis with EDS – qualitative and semi-quantitative results
- Magnification – from 5x to 300,000x
- Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height
- Materials analysed – solid inorganic materials including metals and minerals.

Fig no:3.7. SEM INSTRUMENT

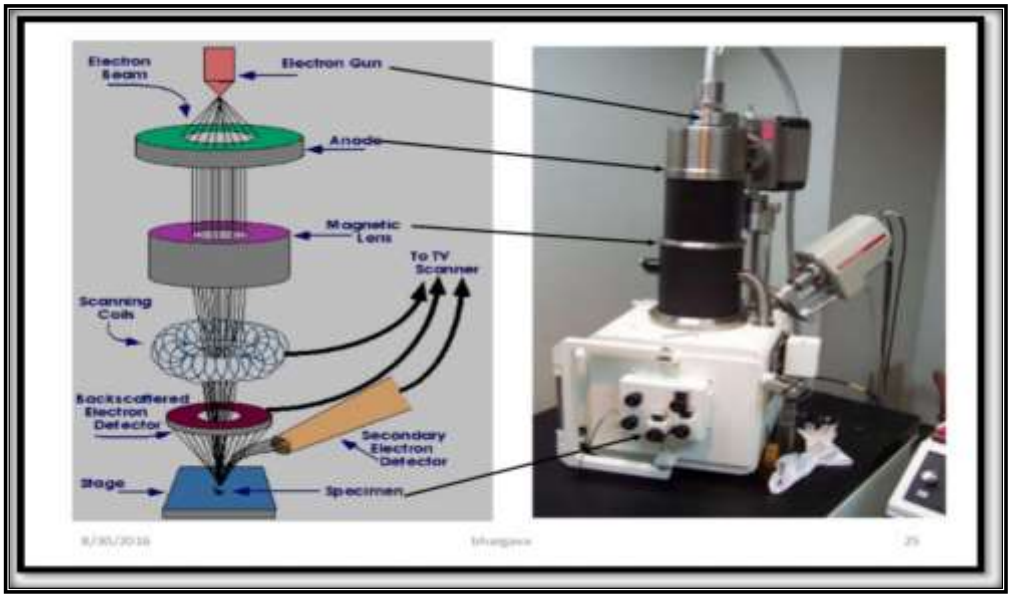


Fig.no.3.8 SEM MECHANISM

THE SEM ANALYSIS PROCESS

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam was focused through a probe towards PP. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include:

- Secondary electrons
- Back scattered electrons
- Characteristic x-rays light
- Specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

4.3. TOXICOLOGICAL STUDIES

4.3.1. ACUTE ORAL TOXICITY – OECD GUIDELINES – 423

INTRODUCTION:

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Acute toxicity study was carried out as per OECD (Organization for Economic Co - operation and Development) guideline-423.

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under CPCSEA (Approval no: **02/321/PO/Re/S/01/CPCSEA dated 12/10/2018)** at C.L.Baid Metha college of Pharmacy, Thuraipakkam, Chennai.

PRINCIPLE:

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing was needed − dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes [84].

METHODOLOGY

Selection of animal species:

- The animal models used in this study were albino animals.
- Healthy Female albino animals weighing 150-250gm were obtained from the Animal house of King's institute, Guindy, Chennai.
- Females should be nulliparous and non-pregnant.
- Each animal must be around 8 and 12 weeks old at the time of dosing.
- The studies were conducted in the animal house of C.L.Baid Metha college of Pharmacy, Thuraipakkam, Chennai-97.

Housing and feeding conditions:

- Animals were housed under standard laboratory conditions.
- They were maintained in a ventilated room. The temperature in the room should be 22º(+3º).
- The relative humidity should be at least 30% and not exceed 70% (50%-60%).
- Lighting should be artificial; it is maintained as 12 hours light, 12 hours dark cycle.
- Animals were kept in a clean polypropylene cage.
- Animals were fed with standard pellet diet (Biogen Foods, Bangalore) and water and libitum.

Preparation of animals:

All the animals were randomly selected and marked on its fur for its individual identification. They were acclimatized to the laboratory conditions at least one week prior to the commencement of the study.

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the ***Kandankathari Chooranam***.

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

Test Substance	: ***KANDANKATHARI CHOORANAM***
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	**:** 8-12 weeks
Body Weight on Day 0	**:** 200-220gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Number of animals	: 3 Female/group,
Route of administration	: Oral
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC + 3ºC.
Relative humidity	**:** between 30% and 70%,
Air changes	**:** 10 to 15 per hour and
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**:** 14 Days

EXPERIMENT PROCEDURE:

Administration of doses

KANDANKATHARI CHOORANAM prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressiness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study : 48 hours

Evalution : 14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

The animals were observed individually after dosing at least once during the first 30 minutes and periodically during the first 24 hours.

Special attention: First 1-4 hours after administration of drug and it is observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

Mortality:

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study.

Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility and necropsy findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *KANDANKATHARI CHOORANAM* with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique. [85].

4.3.2.REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *KANDANKATHARI CHOORANAM* (*KKC*) ON RATS – (OECD- 407 guidelines)

Test Substance	: ***Kandankathari Chooranam***
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Male -24, and Female-24s)
Age	**:** 6-8 weeks
Body Weight	**:** 150-220gm.
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and Individual marking by using Picric acid
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC + 3ºC.
Relative humidity	**:** between 30% and 70%,
Air changes	**:** 10 to 15 per hour
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**: 28 Days.**

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *KANDANKATHARI CHOORANAM* was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were 100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because the oral route was considered to be a proposed therapeutic route [86].

Preparation and administration of dose

KANADANKATHARI CHOORANAM at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

Twelve rats (Six Male and Six Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Table 1

Groups	No of Rats
Group I Vehicle control (Normal Saline)	12 (6 male, 6 female)
Group II KKC 100 mg / kg	12 (6 male, 6 female)
Group III KKC 200 mg / kg	12 (6 male, 6 female)
Group IV KKC 400 mg / kg	12 (6 male, 6 female)

KKC-Kandankathari Chooranam.

OBSERVATIONS

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight*:*

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality*:*

All animals were observed twice daily for mortality during entire course of study.

Functional Observations:

At the end of the 4^{th} week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.The urine was free from fecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations.

On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes : one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

Blood samples of control and experimental rats were analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations:

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis:

Urine samples were collected on end of the treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy*:*

All the animals were sacrificed on day 29. Necropsy of all animals were carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA

followed by Dunnet's multicomparision test using a computer software programme GRAPH PAD-8 version.

4.4. PHARMACOLOGICAL ACTIVITIES:

4.4.1. Evaluation of Bronchodilator activity:

Overnight fasted guinea pigs were divided into four groups each containing 6 animals.

- Group 1 was treated as control,
- Group 2 received standard drug chlorpheniramine maleate (2 mg/kg).
- Group 3 *Kandankathari Chooranam* 200 mg/kg.
- Group 4 *Kandankathari Chooranam* 400mg/kg.

All the doses were given orally once a day for 5 days. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The pre convulsive time (PCT) was determined from the time of exposure to onset of convulsions. As soon as the PCT were noted, the animals were removed from the chamber and placed in fresh air. Group 2 received Chlorpheniramine maleate. These animals were again subjected to histamine aerosol after 1hour of drug administration and PCT was determined. The protection offered by treatment was calculated by using the formula [87].

Percentage Protection = (1 - T1/T2) X 100 Where,

T1 = the mean of PCT before administration of test drugs.

T2 = the mean of PCT after administration of test drugs.

4.4.2. Evaluation of Antihistamine activity:

Vascular permeability test in rats:

Immediately after an i.v. injection of 1 ml of 1 Evans blue in physiological saline, two sites on one side of the shaved back of animals were injected intradermally with 0.1 ml of physiological saline containing 0.1 µg histamine, contralateral sites were injected intradermally with an equal volume of physiological saline (the control skin areas). *Kandankathari Chooranam* was given orally 30 min in rats prior to the injection of phlogistics. Thirty minutes later, the animals are sacrificed by overdose of anesthesia, and the skin was removed. Exudation of dye was calculated by subtracting the amount

determined in the control skin area and expressed as the mean of two values obtained in each animal [88].

Calculation:

Area of protection = control area – area of exudation of dye

Grouping: Wistar rats were used for the study n=6nos

Group 1----------Control group

Group 2----------Standard drug Cetrizine 20mg

Group 3----------*Kandankathari Chooranam* 200mg/kg

Group 4----------*Kandankathari Chooranam* 400mg/kg

Experimental Procedure

Guinea pig was sacrificed and a segment from ileum (2 cm) was dissected from the terminal ileum and mounted in an organ bath containing Tyrode solution (10 ml) between two stainless steel hooks under 0.5 to 1 g initial tension. The lower hook was fixed at the bottom of the organ bath and upper one was connected to an isotonic transducer. The Tyrode solution composition (pH 7.4) was NaCl-8.0, KCl-0.2, $CaCl_2$-0.2, $MgCl_2$-0.1, $NaHCO_3$ -1.0, NaH_2PO_4-0.05, and Glucose-10.0gms/liter.

It was continuously aerated and maintained at 37 ± 0.5ºC. The equilibrium period was 60 min and the bath solution was refreshed every 15 min. After equilibrium period, a dose response curve for histamine in variant molar concentrations, by maintaining 45 min time cycle was taken separately. Results are provided in the table

Statistical Analysis

Ileum contractions induced by agonist were assumed as 100% and reductions induced by test drug calculated. Percentage of ileum contraction was expressed as mean ± SEM. Results were analyzed using one-way analysis of variance (ANOVA). Probability value less than 0.05 were considered as significant. Results are discussed in table.

Method:

After the end of sub-acute toxicity study, the intermediate dosage groups of animals were sacrificed and organs such as liver and kidneys were excised out and analyzed for oxidative stress markers. The concentration of oxidative stress markers such as Lipid peroxide, Glutathione, Glutathione peroxidase and Catalase were analyzed. Lipid peroxides (Thiobarbituric Acid Reactive Substances – TBARS) in tissues were assayed by the method of Yagi (Yaki K, 1978). The colour formation with Thiobarbituric acid (TBA) was used as index. Reduced glutathione (GSH) was estimated by the method of Ellman in which yellow colour developed when dithionitro-bis-benzoic acid (DTNB) added to the compounds sulfhydryl groups (Ellman GL, 1959). Glutothione peroxidase (GPx) estimated by the method of Rotruck et al, 1973 in which H2O2 reduced to water whereas organic hydroperoxides reduced to alcohol at the expense of GSH (Rotruck JT et al. 1973). The activity of Catalase (CAT) was determined by the method of Sinha (Sinha AK, 1972). In this assay, Dichromate in acetic acid heated in the presence of hydrogen peroxide converted to perchromic acid and then to chromic acetate. The formed chromic acetate was measured at 620 nm.

4.4.3. Evaluation of Antioxidant activity (In-Vitro Model):

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)

The radical scavenging activity of *KKC* extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H- A) can be written as,

$$(DPPH) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mm DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different volumes (1.25-20µg/µl) of *KKC* extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mm) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control.

The % radical scavenging activity of the *KKC* extracts was calculated using the following formula,

$$\text{Percentage of inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug ***Kandankathari Chooranam***. The studies includes Literary collections, Organoleptic character, Physicochemical and Phytochemical analysis, Microbial load, Instrumental analysis, Toxicological studies and Pharmacological studies. The drug ***Kandankathari Chooranam*** has been selected for Bronchodilator activity in reference with the text ***"Agathiyar Attavanai Vagadam".***

Discussion on review of literature:

Literary collections about the drug from various text books were done. Siddha literatures related to ingredients of the drug bring the evidence and importance of its utility in treating Bronchial Asthma.

Botanical aspect explains the identification, description, active principle and medicinal uses of the Ingridents.

Gunapadam review brings the effectiveness of the drug in treating Bronchial Asthma.

Pharmaceutical review describes about the *Chooranam* and its properties.

The Pharmacological review explains about the methodology of Bronchodilator Activity and Anti histamine activity.

Modern and Siddha aspect of the disease was also reviewed.

Standardization of the test drug:

Standardisation of the drug is more essential to derive the efficacy and the potency of the drug by analysing it by various studies. Following are the results of physicochemical and phytochemical analysis. Physical characterisation and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug was derived. Its result has been tabulated and the interpretation was made below. Thus it give a complete justification to bring the effectiveness of the trial drug *Kandankathari Chooranam.*

The extensive review on botanical aspect gave information about the microscopical characters, macroscopical characters, medicinal uses, constituents and the importance of the herbs in detail. Most of the herbs included in this preparation exhibit bronchodilator effect which is useful in the treatment of bronchial asthma.

Solanum xanthocarpum exhibits expectorant and anti-inflammatory actions. It produces diminishing effect in the intensity of cough and dyspnoea [(89)].

Zingiber officinale relaxes the airway smooth muscle and potentially serve as novel bronchodilator [(90)]. It also possesses anti-oxidant activity [(91)].

Piper nigrum has contains a pungent alkaloid ‘‘Piperine’’ which is known to possess many pharmacological actions like antioxidant, anti-asthmatics, anti-inflammatory, anti-diarrheal, antispasmodic, immunomodulatory activity. All these activities primarily favours the treatment of bronchial asthma [(92)].

Piper longum also have Piperine, which is the prime constituent of fruit which have significant anti - inflammatory and antioxidant activity [(93)]. It also possesses expectorant effect and is commonly used for Bronchial asthma [(94)].

Toddalia asiatica is used for treatment of cough, fever and epilepsy and also as and expectorant, analgesic, diaphoretic and anti-inflammatory [(95)].

Discussion on pharmacological aspect:

- The pharmacological aspect of the drug says about their mode of action and the side effects which were used worldwide since ancient times.

- The current pharmacological methods available for carrying out the Bronchodilator studies were explained clearly and the suitable In-vivo models carry out the activities were discussed.
- Result from the pharmacological study denotes the effects of *Kandankathari Chooranam (KKC)* showed the promising effects in treating lung diseases.

Discussion on Pharmaceutical review:

- This review explained the preparation of *Chooranam* in detail including the purification of raw drugs, methods of manufacturing of *Chooranam* and the *Siddha* parameters for the standardization of *Chooranam.*
- The powdered drugs were filtered through the white cloth so as to reduce the size of the particle in turn which enhances the bio-availability.
- The shelf life of the drug is improved by proper purification methods and preservation.

Discussion on Materials and Methods:

- The preparation of the drug was done carefully so as to achieve the highest potency. *Chooranam* are fine, dry powders of drugs. The term *Chooranam* may be applied to the powder of single drug or a mixture of two or more drugs.
- On purification (pittaviyal), the weight of the *Chooranam* is different from the exact value but not from the mean value when calculated.
- The *Chooranam* are also subjected to *Siddha* parameters of the testing like,

  Chooranam tends to be amorphous,

  It should be never damp,

  The fitness of the sieve should be 100 mesh or still finer.
- The standardization of the drugs was achieved through various procedures like analyzing the organoleptic characters, physico-chemical characters, elements present in the drug and the results and discussion of standardization parameters are described below.

ORGANOLEPTIC CHARACTER:

Table: 2 Results of Organoleptic characters

S.No	Parameter	Results
1.	Colour	Brown
2.	Odour	Characteristic odour
3.	Taste	Pungent & Sweet
4.	Sense of touch	Fine powder
5.	Size	Completely pass through sieve no 88

PHYSICOCHEMICAL ANALYSIS:

Table: 3. Results of Physicochemical Analysis:

S.NO	PARAMETERS	PERCENTAGE
1.	PH	6.08
2.	Loss on drying	0.99%
3.	Total ash value	5%
4.	Acid insoluble ash	1%
5.	Water soluble ash	1%
6.	Water soluble extraction	30.4%
7.	Alcohol soluble extraction	10%

8.	Solubility	
	1.Distilled water	Soluble
	2.Ethonal	Soluble
	3.Chloroform	Soluble

The physicochemical analysis of the drug (*KKC)* result reveals pH, Loss on drying total ash value, Acid insoluble ash and Water soluble ash. The interpretation of the result were given below.

Interpretation:

1. pH:

It is a measure of hydrogen ion concentration. It is the measure of the acidic or alkaline nature. 7.0 is neutral, above 7.0 is alkaline and below is acidic.

The pH of the drug *Kandankathari Chooranam* is 6.08 which is weak acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach.[96].

2. Moisture (Loss on drying):

The total amount of volatile content and moisture present in the drug was established in loss on drying. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life. Loss on drying of *Kandankathari Chooranam* is 0.99% [97]**.**

3. Total Ash:

Ash constitutes are the inorganic residues obtained after the complete combustion of a drug. Thus Ash value is a valid parameter to describe and to assess the degree of purity

of a given drug. Total ash value of plant material indicated the amount of minerals and earthy materials present in the drug. The total ash value of *Kandankathari Chooranam* is 5% which determine the absence of inorganic content.

4. Acid insoluble ash:

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluable ash value of *Kandankathari Chooranam* is 1%. Thus the medicine is highly effective.

5. Water soluble ash:

Water-soluble ash is the part of the total ash content, which is soluble in water. Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanism. Water soluble ash value of *Kandankathari Chooranam* is 1%. Thus the medicine is highly effective.

6. Solubility:

Solubility is the major factor that controls the bioavailability of a drug substance. It is useful to determine the form of drug and processing of its dosage form.

The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability .[98].

KKC is soluble in major solvents and sparingly soluble in some solvents proves that its efficiency of solubility in the stomach indirectly, increasing the bio availability.

PHYTOCHEMICALS ANALYSIS

Table: 4. Results of Phytochemicals screening test:

S.NO	PHYTOCHEMICALS	TEST NAME	H2O EXTRACT
1.	**Carbohydrates**	Molisch's test	+Ve
2.	**Glycosides**	Modified Borntrager's test	+Ve
3.	**Saponins**	Foam test	+Ve
4.	**Flavonoids**	Alkaline reagent test	+Ve
5.	**Diterpenes**	Copper acetate test	+Ve
6.	**Gum and Mucilage**	Extract + Alcohol	+Ve

The above stated phytochemical properties for the given sample certified to be present.

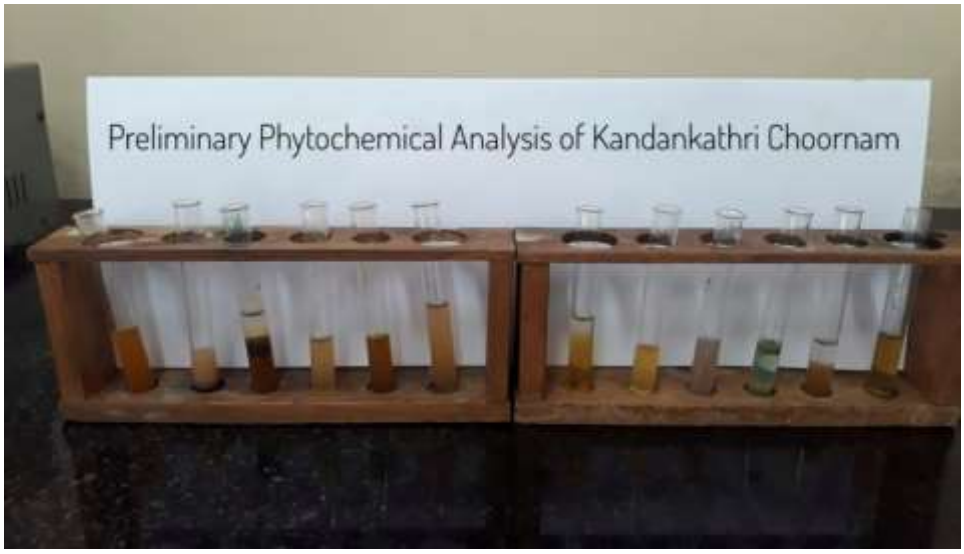


Fig.no.4. Preliminary phytochemical analysis of *Kandankathari chooranam*

The phytochemical analysis of the drug (*KKC*) result reveals Carbohydrats, Glycosides, Saponins, Flavanoids, Diterpenes and Gum and Mucilage. The interpretations of the result were given below.

Interpretation:

Carbohydrate:

- Carbohydrate contains plenty of antioxidants, vitamins and fiber that are ecessary for our health.Carbohydrates provide energy for physical activity and functions of the body. Repair of epithelial tissue injury in asthma was made by carbohydrates.
- Carbohydrates plays an important role in storage of glucose. It regulates the blood glucose level and provides energy to the body.
- Carbohydrates help in fat metabolism. It plays an important role in homeostasis.
- Carbohydrates help us to fight inflammation and cancer, improve our digestive system, heart and bone health. [99].

Glycosides:

- Glycosides inhibit eosinophil accumulation in tissue and allergic inflammation.
- Many plants store chemicals in the form of inactive glycosides, such plant glycosides are used as medications. Glycosides have antibacterial activity, so they protect our body from bacteria and infectious diseases.
- Glycosides increase the intestinal motility. So it produces laxation. They can be Effectively used for preventing or treating allergic diseases associated with inflammation and eosinophil accumulation such as COPD, Bronchial asthma and Allergic rhinitis [100].

Saponins:

- It has anti-spasmodic, anti-inflammatory, expectorant and anti-oxidant property.
- Saponins quicker the expulsion of mucous from the lung.
- In the digestive tract, saponins produce an emulsification of fat soluble molecules. Saponins bind with bile acids and helps to eliminate them from the body, preventing cholesterol from being reabsorbed.
- Saponins can boost the immune system, have an antioxidant effect and may even support bone strength.[101].

Flavanoids:

- It is the most important group of polyphenolic compounds in plants.
- Flavonoids can exert their anti-oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation.
- Flavanoids are immunomodulator. It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.[102].

Diterpenes:

- Diterpene has an Anti-oxidant, Anti-inflammatory and mucolytic activity.
- Diterpenes helps to cure hypertension. It also has tumour inhibitory properties as well as a stimulating effect on the immune system.
- Suppress the inflammatory response.
- They are often expectorant.[103]

Gum and Mucilage:

- Gum is used as a bulk laxatives and mucilage are used for their demulcent properties for cough suppression.[104].

A synergistic effect of all these Carbohydrate, Glycosides, Saponin, Triterpenes and Flavonoids increases the potency of the drug against Bronchial asthma.

High Performance Liquid Chromatography (HPLC):

HPLC analysis were done. HPLC analysis performed with *Kandankathari Chooranam* revealed the pressence of following compounds:

Table:5

S.No	PARAMETERS	METHOD	UNITS	RESULTS
1.	Total polyphenol as gallic acid equilant	Indian pharmacopoeia 2014	Mg.100g	0.09
2.	Total flavonoids as quercetin equivalent	TNTH/STP/FOOD/110	Mg/100g	18.15
3.	Total alkaloids	TNTH/STP/FOOD/426	Mg/100g	1.93
4.	Total tannin as acid equivalent	AOAC20th Edn.2012, 955.35	Mg/100g	0.98

HPLC analysis reveals the pressence of polyphenols, Flavanoids, Alkaloids and Tannins.

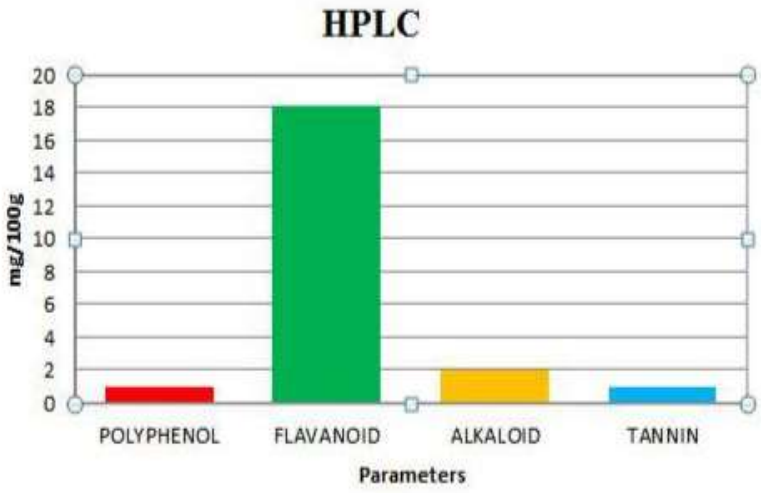


Chart no.1

Interpretation:

- Polyphenols are the member of very large family of plant derived compounds which had the anti lipidogenic effect. This is mainly due to reduce fatty acid and triglycerol synthesis, increased in fatty acid oxidation and reduction of oxidative stress and inflammation.
- Polyphenols are biomolecules which produce Bronchodilator effects which reduce the fat accumulation, mainly by reducing lipogenesis and by increasing fatty acid oxidation and decrease oxidative stress and inflammation.[105].
- Flavanoids a group of plant compounds which have the beneficial effects of Bronchial asthma.
- Flavanoids prevent Hepatosteatosis by increasing fatty acid oxidation in liver. They can also reduce caloric intake and decrease the body weight and fat deposition in viseral tissues.
- Flavanoids are the unique antioxidant. It also correct dislipidemia and blood pressure[106].
- Tannins and alkaloids contains anti-oxidant effect which produce many essential effects in protecting the body.

s

BIO CHEMICAL ANALYSIS:

Table: 6.Results of basic radicals studies:

S.no	Parameter	Observation	Result
1	Test for Potassium	Yellow colour precipitate	Positive
2	Test For Magnesium	White colour precipitate	Positive
3	Test for Iron (Ferrous)	Blood red colour	Positive
4	Test For Calcium	White Precipitate	Positive

The basic radical test reveals the presence of Potassium, Magnesium, Iron and Calcium. The interpretation of the result were given below.

Interpretation:

Presence of these traces of minerals play an important role in the functioning of various enzymes in biological system and also have immunomodulatory function and hence the susceptibility to the course and the variety of viral infections.

Potassium:

- Potassium levels may be an indicator of impending lung problems.
- Potassium is absorbed through the small intestine. Severe lack of potassium can disturb the lung function and if potassium level falls below 30% to 40% causes Respiratory Disorders.
- Potassium is important for maintaining the integrity of cell membranes and functions as a vital electrolyte.[107].

Magnesium:

- Magnesium ions are responsible for bronchodilator and anticholinergic action which helps in acute asthma.
- It also helps to regulate blood glucose levels and aid in the production of energy and protein[108].

Iron:

- Presence of iron in the drug has increased haemoglobin concentration in the blood.
- It enhances the arterial oxygen level.
- The drug enhances oxygen supply, promotes the normal ventilation of the lungs and reduces the dyspnea.

- It also reduces airway hyperactivity and eosinophilia.[109].

Calcium:

- Calcium is important for normal muscle contraction and blood vessel structure.
- Calcium is a cell signaling mineral, which means it plays a vital role in cell-to-cell communication. Contraction and expansion of respiratory muscles allows the lungs to breathe in and out.
- Calcium is crucial for muscle movement and it helps an individuals to maintain normal breathing rhythm.

So Calcium, Potassium, Magnesium and Iron of this drug help to achieve its activity on bronchial muscles.[110].

Table: 7. Results of acid radical studies:

S.NO	Parameter	Observation	Result
1	Test for Chloride	Formation of white precipitate	Positive
2	Test for Phosphate	Formation of yellow precipitate.	Positive

Interpretation:

The acid radical study of *KKC* shows the presence of **Chloride and Phospate.**

Chloride

- Chloride is needed to keep the proper balancing of body fluids [111)).
- It maintains proper blood volume and pressure [112].
- It plays critical roles in inflammatory airway diseases such as Bronchial asthma and Allergic rhinitis [113].

Phosphate

- Phosphate is a charged particle that contains the mineral phosphorus [114].
- The mineral Phosphorus is primarily used for growth and repair of body cells and tissues[115].
- It reduces the histamine release by activated mast cells [116].

AVAILABILITY OF MICROBIAL LOAD IN *KANDANKATHARI CHOORANAM*

GRAM POSITIVE

Table 8: Organism: *Staphylococcus aureus*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Kandankathiri Choornam (KKC)	Streptomycin (100µg)	26
	250	16
	500	17
	1000	18

14mm- Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

GRAM NEGATIVE

Table: 9: Organism: *E.coli*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Kandankathiri Choornam (KKC)	Streptomycin (100µg)	26
	250	15
	500	16
	1000	20

14mm- Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

Table10: Organism: *Klebsiella pneumonia*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Kandankathiri Choornam (KKC)	Streptomycin (100µg)	25
	250	14
	500	16
	1000	18

14mm- Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

Table: 11 Organism: *Pseudomonas aeroginosa*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Kandankathiri Choornam (KKC)	Streptomycin (100µg)	30
	250	16
	500	18
	1000	24

14mm- Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

FUNGAL LOAD

Table: 12 Organism: *Aspergillus niger*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Kandankathiri Choornam (KKC)	Clotrimazole(100µg)	37
	250	15
	500	16
	1000	18

14mm- Low sensitive, 15mm-Moderate, 16mm-Highly sensitive.

INTERPRETATION :

1. Staphylococcus aureus - Highly sensitive in 250(µg/ml)
2. Escherchia coli - Highly sensitive in 500(µg/ml)
3. Klebsiella pneumoniae - Highly sensitive in 500(µg/ml)
4. Pseudomonas aeruginosa - Highly sensitive in 250(µg/ml)
5. Aspergillus niger - Highly sensitive in 500(µg/ml)

Discussion:

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial and anti-fungal agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *KKC* was compared with Standard drug Streptomycin and Clotrimazole (100µg)/ml disc for the following pathogens, they are *Staphylococcus aureus, Escherchia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Aspergillus niger*. The results represents *KKC* potentially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The findings reveal that the Siddha drug *KKC* have anti-microbial potency against bacterial and fungal pathogens which is used in the treatment of diseases[(117)].

INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red Spectroscopy)

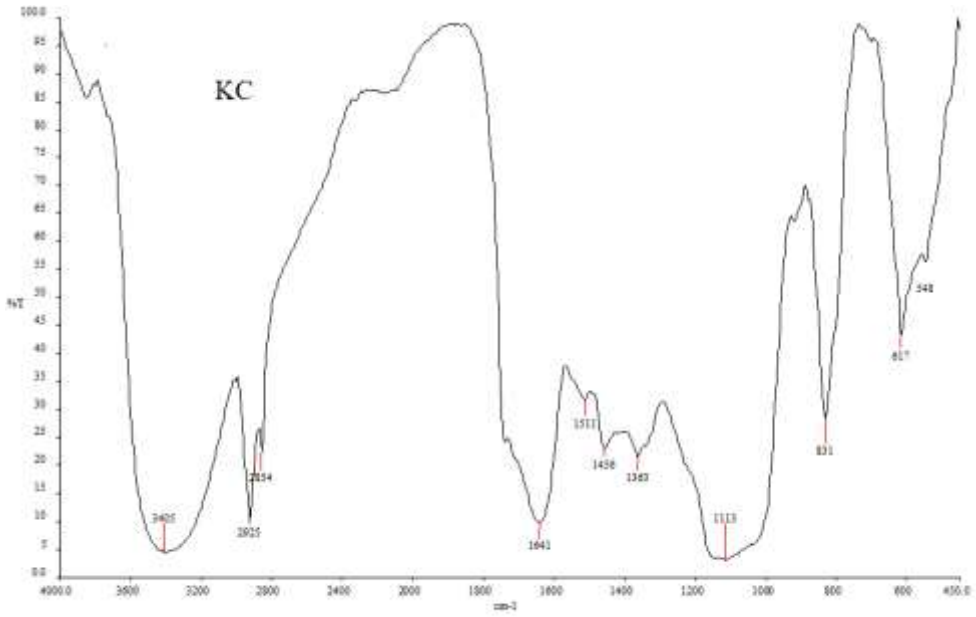


Fig no:5. FT-IR Graph of *Kandankathari Chooranam*

Table 13: FT-IR Interpretation of *Kandankathari Chooranam*

Frequency, cm^{-1}	**Bond**	**Functional group**
3405	O-H Stretch, H-bonded	Alcohols,phenols
2925	C-H stretch, O-H stretch	Alkanes and Carboxylic Acid
2854	C-H stretch, O-H stretch	Alkanes and Carboxylic Acid
1641	-C=C- Stretch	Alkenes
1511	N-O asymmetric stretch	Nitro compounds
1456	C-H bend, C-C Stretch (in ring)	Alkenes, Aromatics
1363	C-H rock	Alkenes

1113	C-O Stretch, C-N Stretch	Alcohols, Carboxylic acid Aliphatic amines
831	C-Cl Stretch, C-H "oop", N-H wag	Alkyl Halides, Aromatics, 1^o, 2^o amines
617	C-Br stretch	Alkyl Halide
548	C-Br stretch	Alkyl Halide

DISCUSSION:

FTIR instrumental analysis was done. The test drug was identified to have 12 peaks. They are the functional groups present in the trial drug *Kandankathari Chooranam*.

The above table shows the presence of Alcohol, Amides, Amines, Acid, Aromatics, Alkyl halides, Alkene, Nitro compounds and alkanes which are represents the peak value.

- OH group has anti-asthmatic effect. It has higher potential towards inhibitory activity against airway inflammation [(118)].
- Amide has mucolytic activity. It makes the mucus less thick and sticky and easier to cough up[(119)].

SEM: (SCANNING ELECTRON MICROSCOPE)

SEM images of *Kandankathari Chooranam*

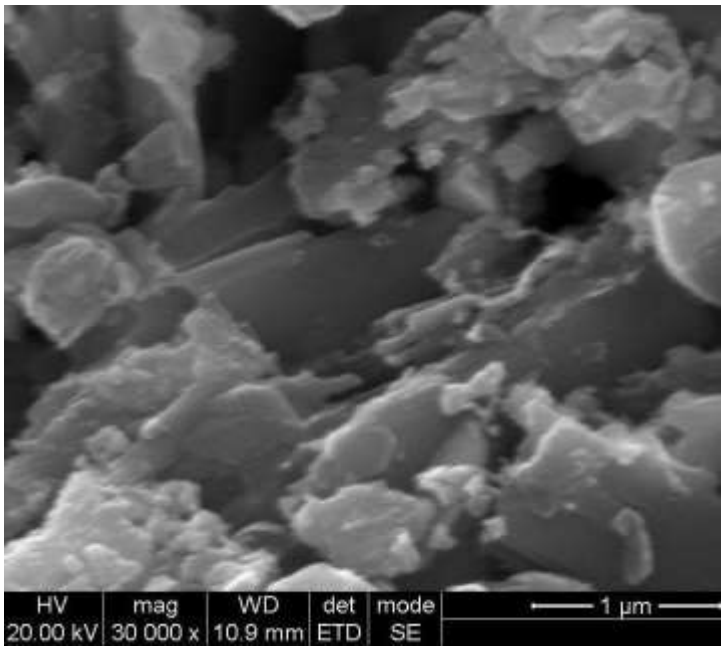


Fig: 6.1. SEM image of 1 µm of *Kandankathari Chooranam*

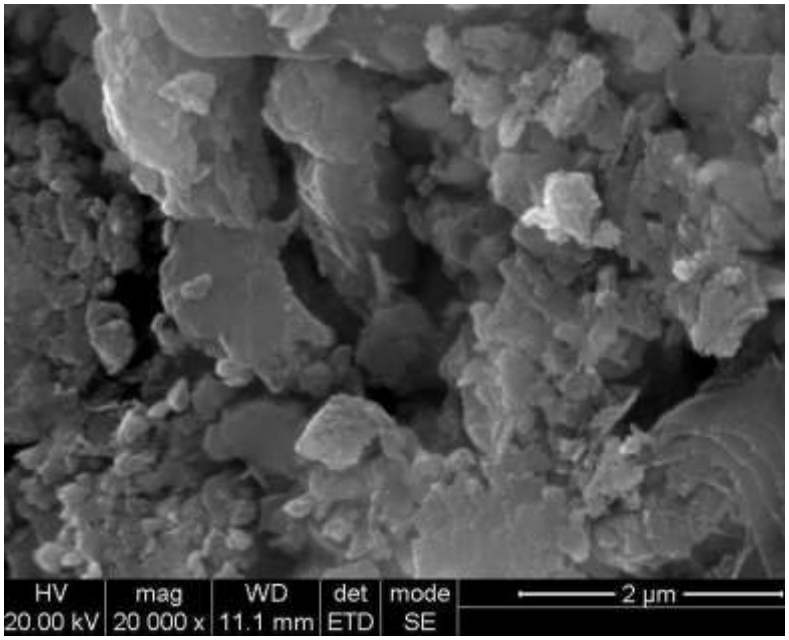


Fig: 6.2. SEM image of 2 µm of *Kandankathari Chooranam*

Interpretation for SEM

- SEM analysis of the test drug *KKC* revealed the presence of Nano and Micro particles of size 79nm, 91nm, 136nm, 196nm and 261nm.
- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100 to 1000nm in diameter. The above SEM studies microscopic resolution showed objects of sizes ranging from 5-2 microns.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting. Nano medicine has its benefit in the treatment for many chronic diseases. Nano particles are smaller in size which enhances the solubility and bioavailability of the drug [120].
- The particles of Nano and micro particles control and sustain the release of drug during the transportation and at the site of localization, alters drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby bio-availability and reduced side effects.

XRD (X-Ray Diffraction studies):

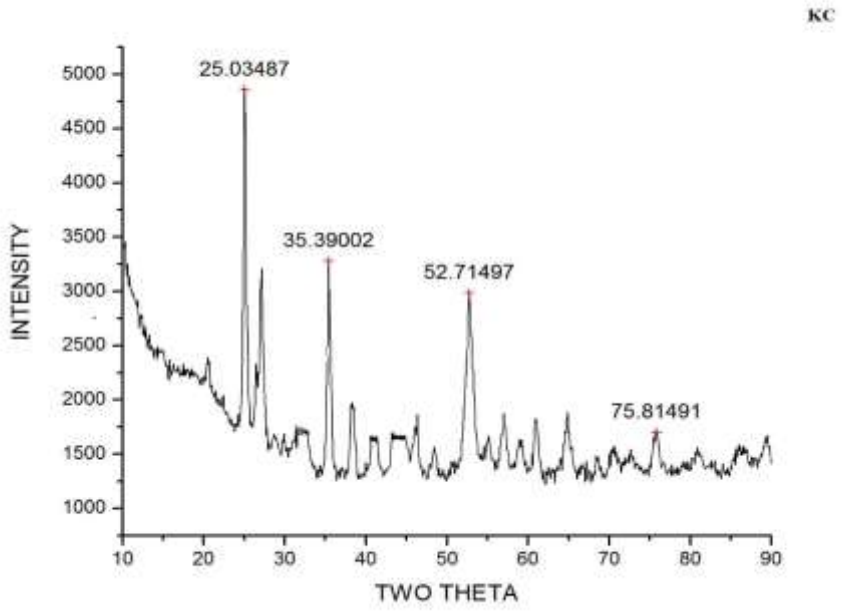


Fig no:7. XRD Graph of *Kandankathari Chooranam*

Interpretation:

The crystallin structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The nano particles may enhance bio absorption of the drug.

XRD pattern of *Kandankathari chooranam* shows the good crystallinity after calcinations process. The major diffraction peaks are identified after XRD analysis *KKC* concluded that Nano crystalline range 25-53nm in association with organic molecules propably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *KKC* act as additional supplement and possibly helps in increase the efficacy of the formulation.[121].

ICP-OES RESULTS OF *KANDANKATHARI CHOORANAM*

Table 14: ICP –OES Results of *Kandankathari Chooranam*

S.NO	Elements Symbol	Wavelength (nm)	Concentration
1.	Aluminum (Al)	396.152	BDL
2	Arsenic (As)	188.979	BDL
3	Calcium (Ca)	315.807	01.190 mg/L
4	Cadmium (Cd)	228.802	BDL
5	Copper (Cu)	327.393	BDL
6	Iron (Fe)	238.204	21.554 mg/L
8	Potassium (k)	766.491	23. 151 mg/L
9	Magnesium (Mg)	285.213	01.154 mg/L
10	Sodium (Na)	589.592	14.100 mg/L
11	Nickel (Ni)	231.604	BDL
12	Lead (Pb)	220.353	BDL
13	Phosphorus (P)	213.617	78.607 mg/L
14	Zinc (Zn)	206.200	01.806 mg/L

DISCUSSION

ICP-OES reveals the concentration of many physiologically important minerals like Ca(01.190mg/L), Fe(21.554mg/L), K(23.151mg/L), Mg(01.154mg/L), Na(14.100mg/L), P(78.607mg/L and Zn(01.806mg/L) in the drug *Kandankathari Chooranam*. The heavy metals like Al, As, Cd, Cu, Hg, Pb and trace element like Ni were below detectable level. This reveals the safety of the drug. Hence the formulation *Kandankathari Chooranam* is extremely safe.

Phosphorus

- Phosphorus is an essential mineral primarily used for growth and repair of body cells and tissues [121].
- Phosphorus is best suited for cough that occurs with asthma.
- It is indicated in the treatment of bronchial asthma [122].

Iron

- Presence of iron in the drug has increased haemoglobin concentration in the blood and enhances the arterial oxygen level. The drug enhances oxygen supply, promotes the normal ventilation of the lungs and reduces the dyspnea.
- It also reduces airway hyperactivity and eosinophilia.

Calcium and Pottasium

- Potassium and Calcium, cell-signaling channel plays a most important role in regulatory part of the respiratory system, breathing rhythm and the body's response to insufficient oxygen levels [123].
- So this drug *KKC* stimulates normal respiratory mechanism.

So Calcium, Potassium, Iron, Sodium, Magnesium, Zinc and phosphorus of this *KKC* drug help to achieve its activity on bronchial muscles.

TOXICITY STUDY RESULTS:

Acute oral toxicity study of *Kandankathari Chooranam* – OECD 423

Dose finding experiment and its behavioural Signs of acute oral

Table:15. Observation of acute toxicity studies

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table: 16 Dose finding experiment and its behavioural Signs of Toxicity for *Kandankathari Chooranam*

Dose Mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Control	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2000mg/kg	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.Alertness 2.Aggressiveness 3. Pile erection 4.Grooming 5.Gripping 6. Touch Response 7. Decreased Motor Activity 8.Tremors 9.Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18.Writhing 19.Respiration 20. Mortality

Table: 17. (Body weight Observation)

DOSE	**DAYS**		
	1	7	14
CONTROL	240.1±65.70	240.3 ± 41.11	240.6 ±02.12
HIGH DOSE	245.3± 6.64	245.7 ±7.42	245.2 ± 2.70
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table: 18. (Water intake (ml/day) of Wistar albino rats group exposed to *KANDANKATHARI CHOORANAM):*

DOSE	DAYS		
	1	7	14
CONTROL	53 ± 3.20	53±6.10	53 ±5.44
HIGH DOSE	54 ±1.30	54±6.70	54 ±5.64
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table :19. Food intake (gm/day) of Wistar albino rats group exposed to *KANDANKATHARI CHOORANAM*

DOSE	DAYS		
	1	7	14
CONTROL	54.03±2.82	54.2±2.96	54.7±8.86
High DOSE	52.6±5.44	52.4±3.20	52.4±2.64
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Interpretation of Acute toxicity studies:

- In the acute toxicity study, the rats were treated with different concentration of *Kandankathari Chooranam* from the range of 5mg/kg to 2000mg/kg.
- This dose level did not produce signs of toxicity, behavioral changes and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.
- However the behavior changes, Body weight, Water intake, food intake does not produce much significant. Thus the results are non-significant.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test the *Kandankathari Chooranam* was found to be nontoxic at the dose level of 2000mg/ kg body weight.

RESULTS OF SUB-ACUTE ORAL TOXICITY 28 DAYS REPEATED DOSE STUDY IN RATS

Table:20. Food (g/day) intake of mice exposed to *KANDANKATHARI CHOORANAM:*

Dose (mg/kg/day)	**Days**				
	1	**7**	**14**	**21**	**28**
Control	1.21±0.1	2.06±0.4	2.99±0.10	3.0±0.31	3.11±0.22
KKC 100	2.01±0.2	2.77±0.3	3.01±0.40	3.0±0.31	3.88±0.40
KKC 200	2.89±0.2	2.77±0.20	2.33±0.31	2.45±0.26	2.97±0.25
KKC 400	3.0±0.4	2.25±0.21	2.28±0.2	2.66±0.28	2.70±0.32
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D*

(One-way ANOVA followed by Dunnett's test)

Table:21. Water (ml/day) intake of rat exposed to *KANDANKATHARI CHOORANAM :*

Dose (mg/kg/day)	**Days**				
	1	**7**	**14**	**21**	**28**
Control	4.1 ± 0.4	4.3±0.2	4.33±3.14	4.5±0.5	4.5±0.4
KKC 100	4.2 ± 0.2	4.1.±0.4	4.0±0.2	4.3±0.3	4.4±0.3
KKC 200	3.9 ± 0.4	4.0±0.5	4.2±0.21	4.6±0.2	4.7±0.5
KKC 400	3.9 ± 0.7	4.7±0.3	4.4±.0.4	4.7±0.4	4.8±.0.4
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table:22. Body weight changes of mice exposed to *KANDANKATHARI CHOORANAM*

Dose (mg/kg/day)	***Days***				
	1	**7**	**14**	**21**	**28**
Control	22.37±3.21	24.14±4.09	28.21±2.17	27.21±5.11	33.32±1.89
KKC 100	23.28±3.21	25.21 ±3.25	26.17 ±2.71	28.12 ±3.41	32.22 ±3.54
KKC 200	24.22 ±2.45	29.45 ±3.65	30.25 ±3.42	32.25 ±2.14	33.25 ±2.34
KKC 400	22.12 ±3.45	28.45 ±3.75	31.48 ±3.25	31.45 ±2.34	33.45 ±3.25
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table :23. Food intake (gm/day) of Wistar albino rats group exposed to *KANDANKATHARI CHOORANAM*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	42±5.21	42.2±4.22	42.8±3.13	43.2±6.72	44±6.80
LOW DOSE	43.6±6.22	43.8±2.42	44.4±1.50	44.5±1.30	44.8±1.12
MID DOSE	45.1±6.70	45.2±2.40	45.6±5.64	46.3±2.40	46.5±1.34
HIGH DOSE	47.4±1.45	47.6±1.34	47.8±2.36	48.1±1.70	48.1±1.62
P value (p)*	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D*

(One way ANOVA followed by Dunnett'stest

Table: 24. Effect of *KANDANKATHARI CHOORANAM* on Haematological parameters in rat

Parameter	Control	100mg/kg	200mg/kg	300mg/kg	P value (p)*
RBC(x 10^6/mm^3)	5.2±0.43	5.16±0.86	6.7±1.2	6.32±0.92	NS
PCV (%)	48.2±1.8	52.4±1.26	56.3±4.6**	52.8±6.4*	NS
Hb (g/dl)	15±0.3	15±1.06	16.2±1.8	15.46±1.8	NS
WBC(mm^3)	8422±183	9567±110**	9724±263**	10568±106**	NS
Neutrophils (%)	18±2	22±1.2**	19±0.9	24±1.6**	NS

Mononuclear cells(%)	78±3	77±2.3	78±1.6	73±0.9**	NS
Eosinophils(%)	3±0.3	2±0.08	1.6±0.12	2.2±0.4	NS
Platelets(x $10^3/mm^3$)	645±6.2	752±15.6**	796.3±17.2**	810±25.4**	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett'stest*

Table:25. Effect of *KANDANKATHARI CHOORANAM* on biochemical parameters in rat.

Parameters	Control	100mg/kg	200mg/kg	400mg/kg
Protein (g/dl)	6.33±0.3	5.72±1.4	6.4±2.4	5.4±0.8
Albumin (g/dl)	2.8±0.2	2.2±0.18	3.2±0.5	2.7±0.24
BUN (mg/dl)	21.33±4.6	20.4±1.6	19.3±1.2	26.4±2.4
Urea (mg/dl)	63±4.3	52±2.2	54±5.2	66.4±3.8
Creatinine (mg/dl)	0.53±0.03	0.52±0.04	0.65±0.007**	0.83±0.04**
Tot.Cholesterol(mg/dl)	111.53±13.17	106±18.2	98.6±7.8	121.4±22.8
Triglycerides(mg/dl)	97.56±14.5	84.3±11.2	96.2±15.4	96.6±9.8
LDH (IU/l)	248.16 ± 59.05	210.83 ± 27.4	211.5 ± 28.78	204.66 ± 50.77 *
Glucose (mg/dl)	113.4±12.2	87.2±9.6**	93.4±10.2**	126.5±13.4*
Total Bilirubin (mg/dl)	0.9±0.08	0.72±0.04**	0.62±0.08**	0.93±0.12
SGOT (U/L)	86.5±5.0	82.4±8.2	97.2±4.6*	91.3±13.3**
SGPT(U/L)	46.5±6.2	36.4±7.3	42.4±5.8	37.4±16.2*

Alkaline phosphatase (U/L)	48.6±7.2	42.6±13.4	52.8±1.2	46.3±3.6
Sodium (mEq/L)	147.3±5.8	132.4±8.2**	148.4±10.4	128.32±7.2**
Potassium (mEq/L)	5.3±0.4	4.6±0.2**	5.1±0.63	4.8±0.59
Chloride (mEq/L)	90.06±0.4	88.04±0.2	91.5±0.63	91.23±0.59

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=12*

DISCUSSION:

The dose selected for the Sub acute toxicity study was 200mg, 400mg/kg of *Kandankathari Chooranam*

All the animals were free of intoxicating signs throughout the dosing period of 28 days. No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment. No abnormal deviations were observed. No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

The weights of organs and the body weight recorded did not show any significant differences in the treatment and the control group indicating that *Kandankathari Chooranam* was not toxic to kidney, liver and spleen.

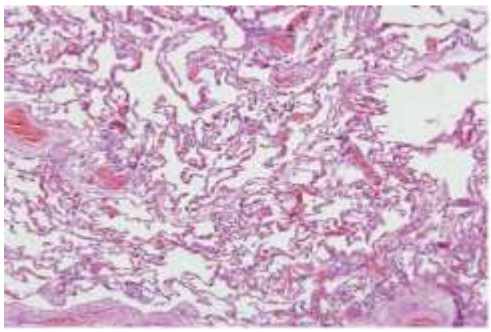
There was no significant changes were observed in haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

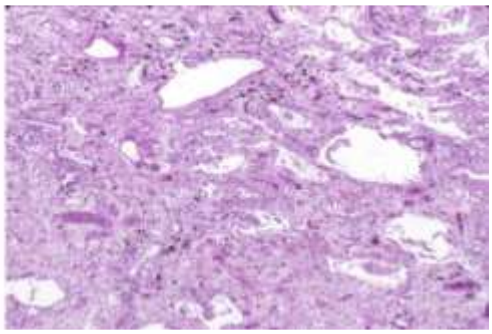
HISTOPATHOLOGICAL STUDIES:

Fig: 8

LUNGS

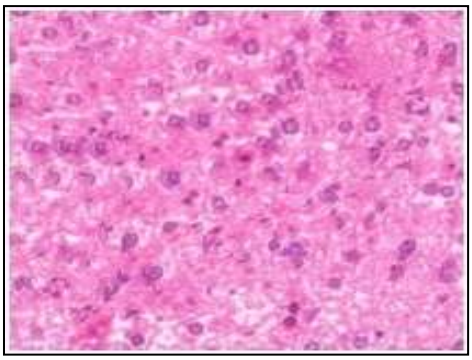
NORMAL **HIGH DOSE**

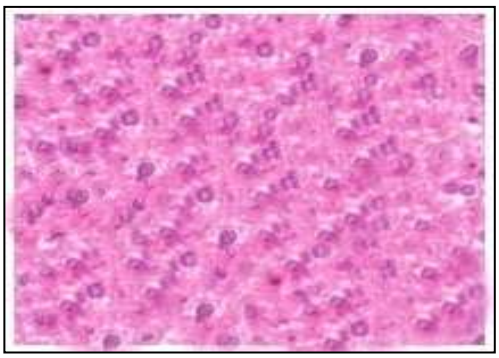




LIVER

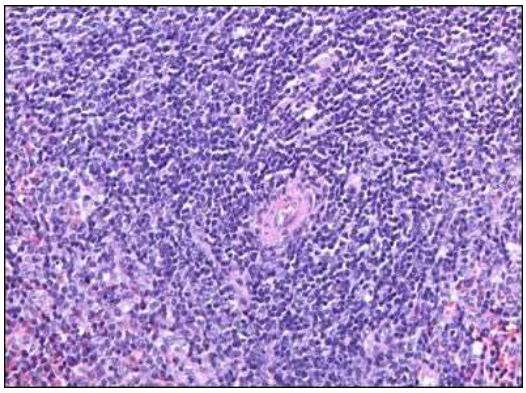
NORMAL **HIGH DOSE**



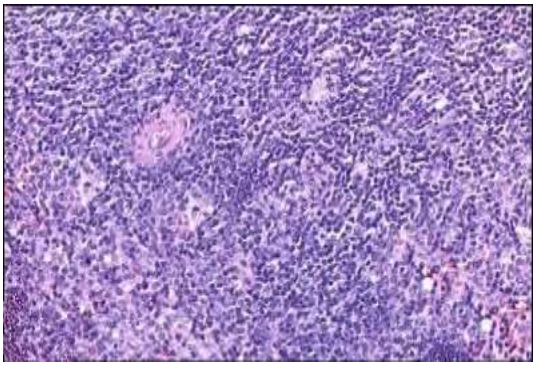


SPLEEN

NORMAL

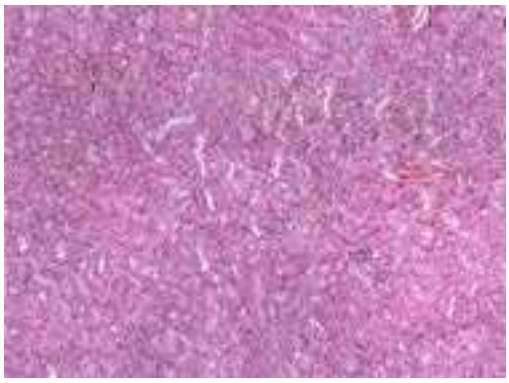


HIGH DOSE

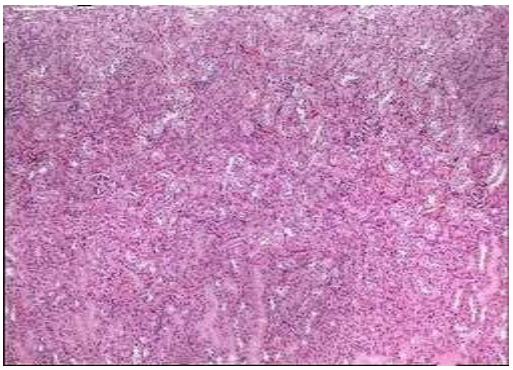


KIDNEY

NORMAL



HIGH DOSE



DISCUSSION:

Histopathological studies were carried out on lungs, liver, kidney and spleen recorded. Blood samples for haematological and blood chemical analyses were taken from common carotid artery.

All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

The acute and sub-acute toxicity studies of *KKC* drug produced some significant changes but the values were found within normal limits. So the drug was nontoxic and safe. It did not produce any adverse effect. So hopefully it could be used for human beings.

The histopathology studies of acute and sub-acute toxicity shows that there is no toxicological abnormality seen in the vital organs after administration of the test drug *Kandankathari Chooranam*. Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

4.4 PHARMACOLOGICAL STUDY

BRONCHODILATOR ACTIVITY

Table: 26. Bronchodilator activity of *Kandankathari Chooranam*

S. No	Group	Onset of Convulsion in sec.	% protection
1	Control	91.45±0.093	--
2	Standard (Chlopheniramine maleate)	1054.2±2.15*	93.6
3	*Kandankathari Chooranam* (200mg/kg)	262.3±1.76**	69.34
4	*Kandankathari Chooranam* (400mg/kg)	456.4.±1.18**	81.32

*Values are mean ±S.E.M. (n=6) *p<0.01, **p<0.001compared with the control animals*

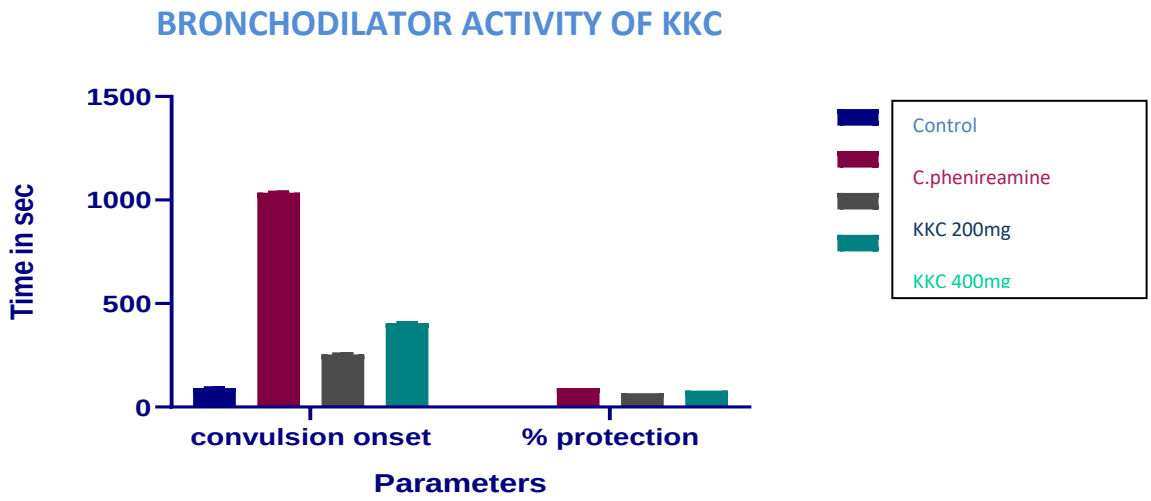


Chart no. 2. Bronchodilator activity of *Kandankathari Chooranam*

DISCUSSION: From the above results, we can confirm that *KKC* possesses bronchodilator activity nearer to fifty percentage when compared with Chlorpheniramine maleate as a standard drug.

ANTI-HISTAMINE ACTIVITY:

Table: 27. Anti-Histamine effect of *Kandankathari Chooranam*

S.No	Grouping	Area of Protection From exudation of Dye in mm
1.	Control	456.94± 1.66
2.	Cetirizine (STD)	139.11± 0.99**
3.	*Kandankathari Chooranam* 200mg	198.02± 3.82
4.	*Kandankathari Chooranam* 400mg	154.61± 0.92*

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control, N = 6*

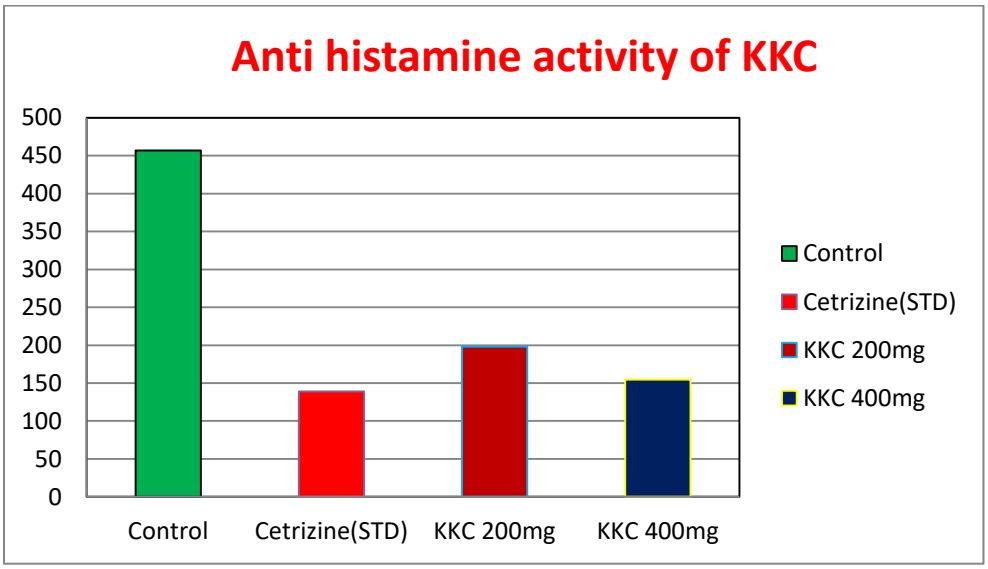


Chart no.3. Anti-Histamine activity of *Kandankathari Chooranam*

DISCUSSION

Mediators like histamine, serotonin and acetylcholine are implicated in various ways in the pathogenesis of Asthma. Histamine is the most implicates mediator in broncho constriction that accompany asthma although the role of serotonin in asthma is uncertain. *KKC* inhibited the histamine induced bronchospasm (vascular permeability) in rats, when compare with cetrizine as standard. Here, KK*C* possesses the Anti-histamine activity.

ANTI OXIDANT ACTIVITY:

Table: 28. DPPH Assay of *Kandankathari Chooranam*

Sample Concentration mg/dl	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
CONTROL	0.3547	1.7983	-	0.00
12.5	1.2314	1.4044	20.69	21.90
25	0.9523	1.0782	39.84	40.04
50	0.5029	0.7121	56.33	60.40
100	0.1765	0.2921	66.68	83.75
200	0.0124	0.0692	85.85	96.15

*****µg/ml: microgram per millilitre. Drug:KKC (41.3211µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)*

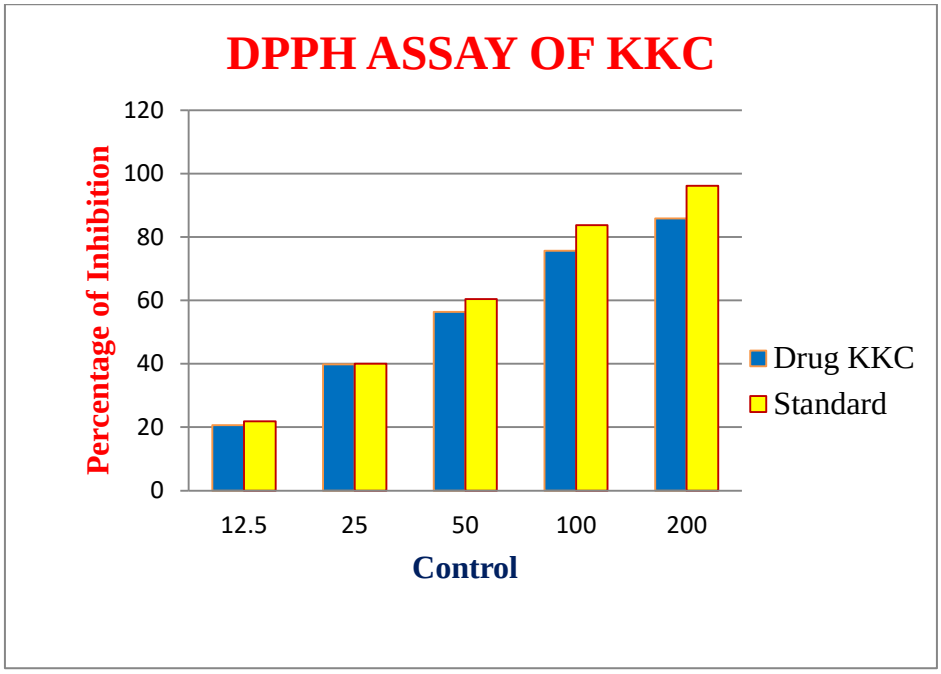
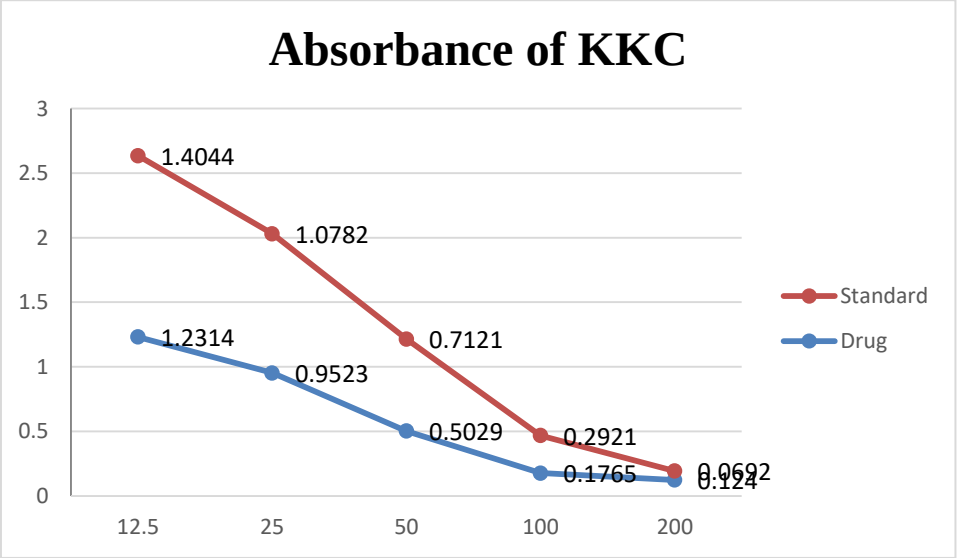


Chart no.4. Anti-Oxidant activity of *Kandankathari Chooranam*



DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *KKC* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2-picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased. In the present study, the *KKC* extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance. In the present study, the extract of *KKC* was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the *KKC* extract was given in (Table No.20). The extract of *KKC* showed the highest DPPH scavenging activity (85.85%) at 200µg/ml and the lowest percentage of inhibition (20.69%) at 12.5µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (96.15%) at 200µg/ml and the lowest percentage of inhibition (21.90%) at 12.5µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and *KKC* extract. The *KKC* extract has more or less equal DPPH scavenging activity when compared to the standard. From the present study, it was concluded that the *KKC* extract has a marked Antioxidant activity at higher concentrations.

CONCLUSION

GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI-106

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Scientific Validation of Siddha Poly Herbal Formulation of "*Kandankathari Chooranam*" for its Bronchodilator, Anti-Histamine and Anti-Oxidant Activities in Animal models** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Karolin Daisy Rani, M.D(S),** Post Graduate Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date:

Place: Chennai

Signature of the Candidate

Dr. L. ILAVARASI

GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI-106

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled "**Scientific Validation of Siddha Poly Herbal formulation of "*Kandankathari Chooranam*" for its Bronchodilator, Anti-Histamine and Anti-Oxidant Activities in Animal models** is submitted to The Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D(Siddha) is the bonafide and genuine research work done by **L.Ilavarasi** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Date: **Seal &Signature of the Guide**

Place: Chennai **Dr. R. KAROLIN DAISY RANI, M.D(S)**

GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI-106

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific Validation of Siddha Poly Herbal formulation of "Kandankathari Chooranam" for its Bronchodilator, Anti-Histamine and Anti-Oxidant Activities in Animal models** is a bonafide work carried out by **L. Ilavarasi** under the guidance of **Dr. R. Karolin Daisy Rani M.D(S),** Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Chennai - 106.

Seal & Signature of the HOD

Dr.R.Meenakumari, M.D(S)

Date:

Place: Chennai

Seal &Signature of the Principal

Dr.R.Meenakumari, M.D(S)

Date :

Place: Chennai

6. CONCLUSION

The trial drug *Kandankathari Chooranam* was selected from the classical Siddha literature, "*Agathiyar Attavanai Vagadam*" for the evaluation of safety and efficacy of the drug in *Swasakasam* (Bronchial asthma).

The trial drug was identified and authenticated by the botanist and experts of Department of Gunapadam, GSMC Chennai. After the purification of the raw drugs by Sarakku Suthi Sei Muraigal, the trail drug *Kandankathari Chooranam* was prepared according to the Siddha classical literature. The purification process of this drug possible to eliminates their toxins and increases its efficacy and the grinding process of this drug helps to change the particle size of the drug for its better bio availability.

Phytochemical analysis of the drug shows presences of Glycosides, Saponins, Carbohydrates, Flavonoids, Diterpenes and Gum and Mucilage. Plant steroids are important to cure the chronic inflammatory diseases like Bronchial Asthma. Glycosides inhibit eosinophil accumulation in tissue and allergic inflammation. Repair of epithelial tissue injury in asthma was made by carbohydrates. Saponin quickens the expulsion of mucus from the lungs.

Biochemical analysis of basic radicals confirms the presence of Iron, Zinc, Potassium and Magnesium. Iron enhances oxygen supply and promotes the normal ventilation of the lungs and reduces the dyspnea. Magnesium ions are responsible for bronchodilator and anticholinergic action which helps in acute asthma.

Zinc and potassium cell-signaling channel plays almost important role in regulatory part of the respiratory system. So, this drug stimulates normal respiratory mechanism.

Biochemical analysis of acid radicals shows the presence of Chloride, Phosphate. Chloride plays critical roles in inflammatory airway diseases such as Bronchial asthma. Phosphate reduces the histamine release by activated mast cells.

Instrumental analysis FT-IR results showed presence of Alcohol, Amide, Amine, Acid, Aromatic, Alkyl halides, Alkene, Ether and Alkane groups. Alcohol group has anti-asthmatic effect. It has higher potential towards inhibitory activity against airway inflammation. Amide has mucolytic activity. It makes the mucus less thick and sticky and easier to cough up.

SEM picture represents Nano and micro particle varying sizes from 79nm to 261nm. It represents the drug is more absorbable and easily to reach the cell. The micro and Nano particles present in the drug results in increased drug therapeutic efficacy thereby bio-availability and reduced side effects.

ICP-OES results show the presence of Phosphorus, Iron, Sodium, Calcium and Potassium. Phosphorus is best suited for cough that occurs with asthma. So, it is indicated in the treatment of bronchial asthma.

Kandankathari Chooranam did not produce any oral acute or sub-acute toxicity in rats. So, the drug was non-toxic and safe. The histopathology studies of acute and sub-acute toxicity show that there is no toxicological abnormality seen in the vital organs after administration of the test drug *Kandankathari Chooranam*.

Kandankathari Chooranam could be conformed as No-Observed-Adverse Effect Level (NOAEL) drug as it acts harmless under normal usage and to be of no toxicological concern.

After evaluate the safety the drug, the Bronchodilator and anti-histaminic property of *Kandankathari Chooranam* is elaborated. So, it can be concluded that this drug inhibits the tone of tracheal and bronchial muscles and thus has a bronchodilator action. It is possible that anti-histamine activity of the *Kandankathari Chooranam* mainly involves inhibiting the histamine induced bronchospasm. The *KKC* extract has more or less equal DPPH scavenging activity when compared to the standard. The *KKC* extract has a marked antioxidant activity at higher concentrations.

From the above scientific evaluation, the author concludes that the drug *Kandankathari Chooranam* is proficient with the new hope in the treatment of Bronchial asthma which is cost effective and has fair preparation method.

SUMMARY

7. SUMMARY

Siddhars improve their long life and virtual power through the modification of *Pranan*. Imbalance in *Pranan* can cause the vitiation of three humours, it can cause bronchoconstriction. Siddhar describes this etiology of symptoms in literature named as a disease *Swasakasam*.

Hence the author conducts the detailed scientific validation of *Kandankathari Chooranam* for Bronchodilator activity, Anti-histamine activity and Anti-oxidant activity.

To collect the information about the drug in various classical Siddha and modern text books, literature was referred. From them, the author came to an idea about the drug and its efficacy on bronchial asthma.

The Phytochemical analysis of the drug evaluates that it contains Carbohydrates, Glycosides, Saponins, Flavonoids, Triterpene and Gum and Mucilage which contributes much in relieving the symptoms of bronchial asthma.

Bio-Chemical analysis of the drug contains Iron, Magnesium, Pottasium, Calcium, Chloride and Phosphate which involves improving normal respiratory function in bronchial asthma.

ICP-OES result shows the presence of Phosphorus, Iron, Sodium, Calcium and Potassium which involves in treating the symptoms of *Kandankathari Chooranam.*

SEM analysis represents the drug contains Nano and micro particles.

The preclinical study showed that the drug has got safe and significant Bronchodilator, Anti-histamine activity and Anti-oxidant activities.

An incredible action of this drug value against the disease of Bronchial asthma has been revealed from this study of *Kandankathari Chooranam.* This must be implicated in the future clinical studies and preclinical chronic toxicity studies.

FUTURE SCOPE

8. FUTURE SCOPE

The trial drug ***Kandankathari Chooranam*** has its own potency in treating Bronchial asthma in animal model which has been established in this study. An incredible action of this drug value against the disease of Bronchial asthma has been revealed from this study of ***Kandankathari Chooranam***. This must be implicated in the future clinical studies and preclinical chronic toxicity studies.

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ANNEXURE